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## European Human Genetics Conference

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**European Meeting on Psychosocial Aspects of Genetics**

**May 21 - 24, 2016**  
**Barcelona, Spain**

**Abstracts**

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## PLENARY LECTURES

## PL1 Opening Plenary Session

## PL1.1

## RAS genes and Human Disease: From Rasopathies to Cancer

M. Barbacid;

Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

RAS genes have attracted a significant deal of attention in biomedical research due to central their role in mediating mitogenic signaling and to their oncogenic activation in at least one third of all human cancers including some of the most frequent and malignant tumor types such as colorectal carcinoma, lung adenocarcinoma and pancreatic ductal adenocarcinoma. More recently, Ras genes have also been implicated in developmental disorders including Costello and Noonan syndromes. In spite of the wealth of information accumulated over the last 30+ years on these genes we still have rather scant information regarding how their misregulation affects human health. More importantly, as of today, there are no efficacious inhibitors to treat those diseases caused by mutations or alterations in RAS genes. Our laboratory has been interested in the development of genetically modified (GEM) animal models that faithfully reproduce the natural history of these diseases and in the identification and subsequent validation of targets with potential therapeutic value. I will describe our GEM models for Costello (H-Ras<sup>G12V</sup>) and Noonan (K-Ras<sup>11V</sup>) syndromes along with our efforts to validate potential therapeutic strategies to correct their developmental defects. I will also describe our new generation of GEM models for K-Ras mutant lung and pancreatic tumors and review the genetic approaches that we are utilizing to validate the therapeutic value of each of the kinases involved in the MAPK signaling cascade, the key pathway involved in K-Ras oncogenic signaling. We hope that these studies will serve to guide the design of future clinical trials to treat people suffering from Rasopathy syndromes as well as cancer patients carrying K-RAS mutant tumors.

## PL1.2

## The Spanish Experience of Retinal Dystrophies

C. Ayuso<sup>1,2</sup>;<sup>1</sup>Madrid, Spain, <sup>2</sup>Fundacion Jimenez Diaz University Hospital (IIS-FJD, UAM), Madrid, Spain.

The retinal dystrophies (RDs) are degenerative diseases involving RPE and/or photoreceptors of the retina and currently without treatment options for the patients. RD may be syndromic or non-syndromic and can affect peripheral, central retina or both. They affect about 1:4,000 individuals and there are more than 270 known causative genes-loci. Mendelian (autosomal recessive, autosomal dominant or X-linked inheritance) and non-mendelian inheritance have been described.

This great clinical and genetic heterogeneity and the need for a genetic diagnosis to help and refine clinical management and guide genetic counseling are a real challenge.

Two decades ago, we started the study of RD in Spain (EsREtNet: Antíñolo, Baiget, Carballo, Millan, Valverde & Ayuso). The modes of inheritance frequency were established (15% Syndromic; and 12% dominant, 39% recessive, 4% Xlinked and 41% sporadic cases and 4% unclassified *Clin Genet* 1995) and the first mutated genes (*RHO*, *CRB1*, *USH2A*, etc) affecting Spanish population were identified, using gene mutation screening approaches (SSCP, dHPLC) or Sanger sequencing.

From 2007, we used array-based primer extension (APEX) technology, with a diagnostic yield from 14% (for autosomal dominant RD; *Blanco-Kelly et al; Mol Vis 2012*) to 54% (for Stargardt cases; *Riveiro et al IOVS 2012*) in RD patients.

In 2011 new genomic analysis technologies, including next generation sequencing (NGS) and chromosome microarrays were implemented. In total 36% of the ≈3000 RD families were characterized and we identified new mutations and genes, and clinical associations.

Molecular characterization allowed us to refine clinical diagnosis, to confirm or change the pattern of inheritance, improving clinical management of RD cases and families. Moreover, we have established very well characterized cohorts of *USH2A*, *NR2E3*, *RHO*, *XLRS*, *CHM* or *ABCA4* patients, useful for natural history of disease studies. Additionally, mutated cases for *RPE65*, *PDEA*, among others can be offered to be included in current clinical trials. At present, a more accurate and efficient algorithms for molecular diagnosis are available for RD patients to improve their clinical care, however we need still to improve the diagnosis rate and fill the gap to obtain effective therapies.

International collaborative efforts for research on molecular, epidemiological and therapeutic aspects are needed to achieve these goals

## PL2 What's New? Highlight Session

## PL2.1

## Genomic landscape of balanced cytogenetic abnormalities in subjects with multiple congenital anomalies

C. Redin<sup>1,2,3</sup>, H. Brand<sup>1,2,3</sup>, R. L. Collins<sup>1</sup>, C. H. Hanscom<sup>1</sup>, V. Pillalamarri<sup>1</sup>, T. Kammin<sup>4</sup>, E. Mitchell<sup>5</sup>, J. C. Hodge<sup>5,6</sup>, S. Schilit<sup>4</sup>, B. B. Curall<sup>4</sup>, S. Pereira<sup>4</sup>, C. Seabra<sup>1</sup>, M. Stone<sup>1</sup>, W. Lawless<sup>1</sup>, D. Luente<sup>1</sup>, C. Antolik<sup>1</sup>, R. Hochstenbach<sup>7</sup>, I. Renkens<sup>7</sup>, E. Brilstra<sup>7</sup>, S. Vergult<sup>8</sup>, B. Menter<sup>9</sup>, S. Janssens<sup>9</sup>, B. Callewaert<sup>8</sup>, A. D'heedene<sup>8</sup>, M. D'hooghe<sup>9</sup>, F. Roelens<sup>10</sup>, J. van de Kamp<sup>11</sup>, A. Nieuwint<sup>11</sup>, P. J. Poddighe<sup>11</sup>, C. van Ravenswaaij-Arts<sup>12</sup>, P. Rump<sup>12</sup>, T. van Essen<sup>12</sup>, J. Freixo<sup>13</sup>, D. Deszo<sup>14</sup>, E. C. Liao<sup>15,16,17</sup>, N. de Leeuw<sup>18</sup>, H. G. Brunner<sup>19,20</sup>, W. Kloosterman<sup>7</sup>, E. C. Thorland<sup>5</sup>, C. C. Morton<sup>3,4,21</sup>, J. F. Gusella<sup>1,2,3</sup>, M. E. Talkowski<sup>1,2,3</sup>,

<sup>1</sup>Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, United States, <sup>2</sup>Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, <sup>3</sup>Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>4</sup>Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Boston, MA, United States, <sup>5</sup>Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States, <sup>6</sup>Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States, <sup>7</sup>Department of Medical Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, Netherlands, <sup>8</sup>Center for Medical Genetics, Ghent University, De Pintelaan 185, Ghent, Belgium, <sup>9</sup>Algemeen Ziekenhuis Sint-Jan, Brugge, Belgium, <sup>10</sup>Algemeen Ziekenhuis Delta, Roeselare, Belgium, <sup>11</sup>Department of Clinical Genetics, VU University Medical Center, De Boelelaan 1117, Amsterdam, Netherlands, <sup>12</sup>University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands, <sup>13</sup>Medical Genetics Service, Central Lisbon Hospital Centre, Lisbon, Portugal, <sup>14</sup>Department of Human Genetics, National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal, <sup>15</sup>Center for Regenerative Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, <sup>16</sup>Division of Plastic and Reconstructive Surgery, Massachusetts General Hospital, Boston, MA, United States, <sup>17</sup>Department of Human Genetics, University of Groningen, Groningen, Netherlands, <sup>18</sup>Department of Genetics, University Medical Center Groningen, Groningen, Netherlands, <sup>19</sup>Department of Human Genetics, University of Groningen, Groningen, Netherlands, <sup>20</sup>Department of Human Genetics, University Medical Center Groningen, Groningen, Netherlands, <sup>21</sup>Department of Human Genetics, University of Groningen, Groningen, Netherlands

Balanced chromosomal abnormalities (BCAs) represent a unique class of genomic variation that involves large rearrangement of the chromosomes. To date their detection has been limited to cytogenetic resolution as most first-tier genetic screening methods are blind to their presence. We defined the genomic landscape of de novo BCAs associated with human congenital anomalies in 235 subjects using whole-genome sequencing. We observed that 22% of all BCAs harbored additional cryptic complexity, ranging from three breakpoints to chromothripsis events involving up to 57 breakpoints. Compared to random expectations, BCAs were more likely to occur between loci in close physical proximity in the nucleus, and their breakpoints were significantly enriched for evolutionarily constrained and embryonically expressed genes. From our convergent genomic interpretation using orthogonal datasets, we predict that the congenital anomaly phenotype was likely attributable to the BCA in at least 30% of subjects. An additional 4% of BCAs disrupted long-range regulatory regions such as topologically associating domains (TADs) resulting in position effects on genes associated with specific clinical manifestations that were compatible with the proband sequenced here. Remarkably, we observed a cluster of six independent translocations that disrupted a TAD and consequently altered MEF2C expression, mimicking the 5q14.3 microdeletion syndrome. These results suggest that de novo BCAs represent a highly penetrant class of genomic variation associated with congenital anomalies, and that nucleotide resolution offers insights into phenotypic prediction from direct gene disruption and alteration of long-range regulatory domains that are likely to be a significant source of causal variation in human disease.

## PL2.2

## Formation and content of novel chromatin domains (neo-TADs) determine pathogenesis of genomic duplications

M. Franke<sup>1</sup>, D. M. Ibrahim<sup>1</sup>, G. Andrey<sup>1</sup>, W. Schwarzer<sup>2</sup>, V. Heinrich<sup>1</sup>, R. Schöpflin<sup>1</sup>, R. Kempfer<sup>1</sup>, I. Jerković<sup>3</sup>, W. Chan<sup>4</sup>, M. Spielmann<sup>5</sup>, B. Timmermann<sup>6</sup>, L. Wittler<sup>7</sup>, I. Kurth<sup>8</sup>, P. Cambiasso<sup>9</sup>, O. Zuffardi<sup>9</sup>, G. Houge<sup>9</sup>, L. Lambie<sup>9</sup>, F. Brancati<sup>10</sup>, A. Pombo<sup>3</sup>, M. Vingron<sup>1</sup>, F. Spitz<sup>2</sup>, S. Mundlos<sup>1,4</sup>;

<sup>1</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>2</sup>European Molecular Biology Laboratory, Heidelberg, Germany, <sup>3</sup>Berlin Institute for Medical Systems Biology, Max Delbrück Centre for Molecular Medicine, Berlin, Germany, <sup>4</sup>Institute for Medical and Human Genetics, Charité, Berlin, Germany, <sup>5</sup>Institute of Human Genetics, University Hospital, Jena, Germany, <sup>6</sup>Bambino Gesù Children's Hospital-IRCCS, Rome, Italy, <sup>7</sup>Department of Molecular Medicine, Pavia, Italy, <sup>8</sup>Center for Medical Genetics and Molecular Medicine; Haukeland University Hospital, Bergen, Norway, <sup>9</sup>Division of Human Genetics, University of the Witwatersrand, Johannesburg, South Africa, <sup>10</sup>Department of Medical, Oral and Biotechnological Sciences, Gabriele d'Annunzio University, Chieti, Italy.

Mammalian genomes are organized in distinctly folded chromatin modules, called topologically associated domains (TADs) that are separated from each other by boundary regions. TADs subdivide the genome into discrete chromatin domains that direct the contacts enhancers can establish with their target genes. How copy number variations interfere with TAD structure and how this might contribute to human disease, is not well understood.

Here, we analyze partially overlapping duplications at the *SOX9* locus, which are associated with a limb malformation (Cooks syndrome), sex reversal or no abnormality in humans. By analyzing patient cells and corresponding mouse models with chromosome conformation capture assays, we show that large duplications spanning a TAD boundary result in the formation of a novel chromatin domain, or neo-TAD. The formation of this neo-TAD explains the divergent phenotypes of the overlapping duplications at the *SOX9* locus. Furthermore, we demonstrate that the duplicated *cis*-regulatory information is functionally isolated and does not influence genes outside the neo-TAD. Instead, the pathogenicity of duplications depends on the combination of genes and *cis*-regulatory information that are contained within the neo-TAD. Taken together, our results show that duplications including TAD boundary regions can result in the formation of novel chromatin domains that are functionally and spatially separated from their genomic neighbors. Moreover, our findings provide a framework for interpreting the pathogenic effect of genomic duplications, frequently detected in patients with congenital malformations, intellectual disability or cancer.

## PL2.3

### Mutations in ACTRT1 and its transcribed non-coding elements lead to aberrant activation of the Hedgehog signaling pathway in inherited and sporadic basal cell carcinomas

*E. Bal<sup>1,2</sup>, Z. Belaid-Choucair<sup>1,2</sup>, H. Kayserili<sup>3</sup>, M. Naville<sup>4</sup>, M. Madrange<sup>1,2</sup>, C. Gordon<sup>1,2</sup>, clinical investigators, G. Gyapay<sup>5</sup>, S. Fraitag<sup>6</sup>, H. Roest Croilius<sup>4</sup>, A. Munich<sup>1,2</sup>, P. Vabres<sup>7</sup>, A. Smahi<sup>1,2</sup>;*

<sup>1</sup>INSERM U1163, Institut Imagine, Hôpital Necker Enfants Malades, Paris, France, <sup>2</sup>Université Sorbonne Paris Cité, Paris, France, <sup>3</sup>Medical Genetics Department, İstanbul Medical Faculty, İstanbul University, İstanbul, Turkey, <sup>4</sup>Institut de Biologie de l'ENS, UMR8197, INSERM U1024, Paris, France, <sup>5</sup>Genoscope (CEA), CNRS UMR 8030, Université d'Evry, Evry, France, <sup>6</sup>Service d'anatomie pathologique, Hôpital Necker Enfants Malades, Paris, France, <sup>7</sup>Service de dermatologie, Centre Hospitalier Universitaire, Hôpital du Bocage, Dijon, France.

Basal cell carcinoma (BCCs) is the most common human cancer, resulting from aberrant activation of Hedgehog signaling pathway. While most cases are sporadic, some forms are inherited, as in Bazex-Dupré-Christol syndrome (BDCS), an X-linked dominant cancer-prone genodermatosis. Studying a series of 6 BDCS families, we found mutations in the ACTRT1 gene, encoding the Arp-T1 protein, in 2/6 BDCS families, and absent expression of Arp-T1 in BDCS tumors and most of sporadic BCCs. High-throughput sequencing revealed germline mutations in non-coding sequences, belong to a new class of enhancers (eRNA), surrounding ACTRT1 in the remaining families. Mutations reduced expression of the specific eRNAs and their enhancer capacity. Using Crispr-Cas9 technology, we demonstrated that induced mutations in these enhancers significantly decreased the expression of Arp-T1 protein. When stably expressed in MDA-MB231 breast cancer and U2OS osteosarcoma cell lines, wild type ACTRT1 reduced cell proliferation and migration. Consistently, xenografts of ACTRT1-MDA-MB231 and ACTRT1-U2OS cells in nude mice demonstrated that ACTRT1 normally inhibits tumor growth. Indeed, transcriptomic analyses revealed that ACTRT1 could modulate the expression of genes involved in regulation of cell cycle progression, cell death and survival or cell migration. Finally, Arp-T1 acts as a tumor suppressor via its direct binding to Hedgehog signaling target genes, thus inhibiting their expression.

Our study identifies a novel mechanism accounting for hereditary and sporadic BCCs, and suggests that ACTRT1 may be potential therapeutic targets in oncology.

This work was supported by the Association pour la Recherche contre le Cancer and Société Française de Dermatologie.

## PL2.4

### De novo mutations MSL3 gene cause a new recognizable syndrome

*A. Bruei<sup>1,2</sup>, I. Aktas<sup>3</sup>, M. Basilica<sup>3</sup>, I. Van der Burgt<sup>4</sup>, J. Morton<sup>5</sup>, S. El Chehadeh-Djebbar<sup>6</sup>, A. Hoischen<sup>6</sup>, C. Gilissen<sup>6</sup>, L. Visser<sup>6</sup>, R. Pfundt<sup>6</sup>, C. Thauvin-Robinet<sup>1,2</sup>, H. Brunner<sup>6</sup>, J. Riviére<sup>1,2</sup>, L. Faivre<sup>1,2</sup>, A. Akthar<sup>3</sup>, J. Thevenon<sup>1,2</sup>;*

<sup>1</sup>Équipe EA42271 GAD, Université de Bourgogne, Dijon, France, <sup>2</sup>FHU-TRANSLAD, Université de Bourgogne/CHU Dijon, Dijon, France, <sup>3</sup>Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany, <sup>4</sup>Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, United Kingdom, <sup>5</sup>Centre de génétique, CHRU de Strasbourg, Strasbourg, France, <sup>6</sup>Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands.

Whole-exome sequencing (WES) has proven to be useful for the identification of the molecular basis of heterogeneous conditions such as intellectual disability with congenital anomalies. A large number of results remain non-conclusive, especially when facing ultra-rare conditions that limit genotype-phenotype correlations. International data-sharing catalyzes the identification of additional cases and allows the delineation of new syndromes. We report on a female patient with developmental delay, moderate intellectual disability, facial dysmorphisms and severe constipation. Af-

ter a negative diagnostic work-up, a proband clinical WES was performed and pinpointed a *de novo* acceptor-splice variant of MSL3 was (NM\_001193270.1:c.1345+1G>T). To assess the relevance of this finding we asked international collaborators for patients with truncating variants of MSL3. Seven unrelated individuals from France, UK, the Netherlands, Germany, USA were identified with *de novo* single-nucleotide variants (4/7), whole-gene *de novo* deletions (2/7) or chromosomal inversion causing a fusion transcript of MSL3 and GAB3 genes (1/7). Clinical findings of patients with point mutation are very similar but less recognizable in patients with chromosomal anomalies. This include speech and walk delay, intellectual disability, neonatal hypotonia, facial dysmorphisms, abnormal limbs, abdominal symptoms and cutaneous anomalies. The MSL3 gene, located on the X chromosome, encodes a subunit of a transcriptional regulator complex responsible of lysine 16 of histone H4 acetylation (H4K16ac). Functional studies revealed a decreasing of H4K16ac mark and complex assembly defects. In conclusion, we delineate a new syndrome of the epigenetic machinery characterized by neurodevelopmental delay and multiple congenital anomalies.

## PL2.5

### Haplotype-assisted noninvasive prenatal diagnosis of common monogenic diseases using massively parallel sequencing of plasma cell-free DNA

*Y. Gao<sup>1</sup>, F. Chen<sup>1,2</sup>, Y. Yuan<sup>3</sup>, Y. Wang<sup>3</sup>, S. Lu<sup>1</sup>, H. Jiang<sup>1</sup>, X. Xu<sup>1</sup>;*

<sup>1</sup>BGI, Shenzhen, China, <sup>2</sup>Section of Molecular Disease Biology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, <sup>3</sup>BGI Clinical Laboratories -Tianjin, Tianjin, China.

#### Introduction

Since 2012, haplotype-assisted noninvasive prenatal diagnosis (NIPD) has been developed in our laboratory, and validated in a number of monogenic diseases using clinical samples from trio families. The purpose of this study was to summarize the performance of this method in different monogenic diseases.

#### Method and Material

Customized panels were designed to capture the coding region and flanking area of disease-associated genes. Genomic DNA from each trio family (father, pregnant mother, and proband child) and plasma cell-free DNA from each pregnant mother was targeted-sequenced using the customized panels. Fetal fraction was calculated with the differently homozygous SNPs in both parents. Parental and fetal haplotype were deduced based on genetic information from the trio family and Hidden Markov Model. Amniocentesis was performed to confirm NIPD results.

#### Result

97 trio families covering 12 monogenic diseases have been tested before receiving prenatal diagnosis (Table). The mutations consisted of point mutations and copy number variants. Mean gestational age of pregnant women while receiving NIPD was 18 weeks. Fetal fraction was between 2.8% to 22.6%, with the mean of 9.1%. 60 pregnancies were identified as disease-affected. So far amniotic fluid was tested for prenatal diagnosis in 70 pregnancies, all consistent to NIPD results.

#### Conclusion

Haplotype-assisted NIPD showed high accuracy in predicting the existence of fetal monogenic disease, and can be used in different diseases and mutation types.

Haplotype-assisted NIPD of monogenic diseases			
Disease	Gene	Trio families	Amniotic fluid available for confirmation
Phenylketonuria (PKU)	PAH	13	12
Methylmalonic acidemia	MMACHC	19	6
Alpha thalassemia	HBA1,HBA2	1	0
Beta thalassemia	HBB	2	2
Hemophilia (A, B)	F9	2	1
Congenital adrenal cortical hyperplasia	CAH	12	12
Spinal muscular atrophy	SMN1	14	13
Duchenne muscular dystrophy	DMD	24	19
Congenital hearing impairment	GJB2	7	2
6-Pyruvoyltetrahydropterin Synthase Deficiency	PTS	1	1
Polycystic kidney disease, infantile type	PKHD1	1	1
Maple syrup urine disease	BCKDHA	1	1
Total		97	70

**PL2.6****Parent-of-origin specific signatures of de novo mutations**

J. M. Goldmann<sup>1</sup>, W. S. W. Wong<sup>2</sup>, M. Pinelli<sup>3</sup>, T. Farrah<sup>4</sup>, D. Bodian<sup>2</sup>, A. B. Stittrich<sup>4</sup>, G. Glusman<sup>4</sup>, L. E. L. M. Vissers<sup>5</sup>, A. Hoischen<sup>5</sup>, J. C. Roach<sup>4</sup>, J. G. Vockley<sup>2,6</sup>, J. A. Veltman<sup>1,7</sup>, B. D. Solomon<sup>2,8</sup>, C. Gilissen<sup>5</sup>, J. E. Niederhuber<sup>2,9</sup>,

<sup>1</sup>Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>Inova Translational Medicine Institute, Inova Health Systems, Falls Church, VA, United States, <sup>3</sup>Telethon Institute of Genetics and Medicine, Naples, Italy, <sup>4</sup>Institute for Systems Biology, Seattle, WA, United States, <sup>5</sup>Department of Human Genetics, Donders Centre for Neuroscience, Radboud University Medical Center, Nijmegen, Netherlands, <sup>6</sup>Department of Pediatrics, Virginia Commonwealth University School of Medicine, Richmond, VA, United States, <sup>7</sup>Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands, <sup>8</sup>Department of Pediatrics, Inova Children's Hospital, Inova Health System, Falls Church, VA, United States, <sup>9</sup>Johns Hopkins University School of Medicine, Baltimore, MD, United States.

**Introduction:** De novo mutations (DNMs) originating in gametogenesis are an important source of genetic variation and can cause human disease. Here, we investigate the properties of DNM on paternal and maternal alleles in order to understand the underlying mechanisms.

**Methods:** We applied whole-genome sequencing to a cohort of 816 parent-offspring trios, yielding in 35,793 autosomal single-nucleotide DNMs. Haplotype assembly could resolve the parental origin of 7,216 DNMs. We compare parental age effects, genomic regions and spectra of paternal versus maternal DNMs.

**Results:** Our results show that the number of DNMs in offspring increases not only with paternal age (0.9 DNMs/year), but also with maternal age (0.24 DNMs/year). The genome regions with highest enrichment for maternally derived DNMs are the known fragile sites FRA8B and FRA16D. We identify parent-of-origin-specific mutation signatures that become more pronounced with increased parental age, with transitions in A.G context being the most biased mutations. Moreover, we find DNMs that are spatially clustered to have a unique mutational signature with no significant differences between parental alleles, suggesting a different mutational mechanism.

**Conclusions:** We find that the different biology of male and female gametogenesis gives rise to distinct mutational patterns. These patterns are valuable to understand physiology and pathology of the human germ line.

## PL3 My vision on genomic medicine

**PL3.1****Our visions of genomic medicine: a dialog**

J. L. Mandel<sup>1,2,3</sup>, M. C. King<sup>4</sup>;

<sup>1</sup>Strasbourg University, IGBMC, Department of Translational Medicine & Neurogenetics, Illkirch, France, <sup>2</sup>CHU Strasbourg, Strasbourg, France, <sup>3</sup>IGMA Genet Diagnostic Lab, Collège de France, Paris, France, <sup>4</sup>University of Washington, Department of Medicine & Genome Science, Seattle, WA, United States.

The invitation from ESHG to both of us to present our thoughts on the current state and the future of genomic medicine bore the risk of having two monologues with undue overlaps or contradictions and too little time for open discussion. We have chosen instead an alternate form of dual presentation of some issues in the development of genomic medicine. We will each speak brief on these questions, in turn, and intermingle our comments with questions and comments from session chairs and from the audience. The topics we propose to address (not necessarily in the final order) are:

- 1) The application of genomics to prediction of risk, to non-invasive diagnosis, and to individualized treatment; given our experience, we think of these issues in the context of cancer;
- 2) Whole genome sequencing for everyone? why? when? For adults, newborns, fetuses, preconception?
- 3) Application of genomic medicine to rare diseases; in particular how to determine pathogenicity and causality given whole genome sequence (e.g. problems of missense variants, non-coding variants, hypomorphic variants). How best to obtain precise and useful natural history for more than 5000 different monogenic diseases? For how many families may specific treatments rather than symptomatic medical care be offered? Meanwhile, what is the place of prevention through prenatal diagnosis? How can one address the risk of uncontrolled eugenism, which already occurs in the context of sex selection in some countries?
- 4) Development of precision medicine for common diseases (beyond cancer, above). What is the current medical value of genotyping and sequencing? How will the value be increased? What is the role of various omics? Of knowledge of gene-environment interactions? How much can we expect new medications based on knowledge of causal genes?
- 5) How can services be made available to everyone? How may direct to consumer testing evolve?

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- 6) How can huge databases be made useful for interpretation of genomic sequence and relationships between health and genotype?

**PL3.2****Our visions of genomic medicine: a dialog**

M. C. King<sup>1</sup>, J. L. King<sup>2,3,4</sup>;

<sup>1</sup>University of Washington, Department of Medicine & Genome Science, Seattle, WA, United States, <sup>2</sup>Strasbourg University, IGBMC, Department of Translational Medicine & Neurogenetics, Illkirch, France, <sup>3</sup>CHU Strasbourg, Strasbourg, France, <sup>4</sup>IGMA Genet Diagnostic Lab, Collège de France, Paris, France.

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- 5) How can services be made available to everyone? How may direct to consumer testing evolve?
- 6) How can huge databases be made useful for interpretation of genomic sequence and relationships between health and genotype?

## PL4 Mendel Lecture

**PL4.1****Mendel Lecture: Epigenetics and Rett syndrome**

A. Bird;

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom.

The word epigenetics excites great interest within biology and beyond, despite uncertainty about its definition. At one level it concerns the significance of the plethora of chemical marks on chromosomes that modulate gene activity (collectively known as the "epigenome"). Prominent among these marks is DNA methylation - a modification that is directly applied to DNA and affects its interaction with proteins. Several clinical disorders involve genes that interpret or lay down the epigenome. For example, the autism spectrum disorders Rett syndrome and MeCP2-duplication syndrome are caused by mutations in the gene for the protein MeCP2. The MeCP2 protein binds to sites on DNA that are chemically altered by DNA methylation and appears to interpret this "epigenetic" mark to affect gene expression. Clues to the function of MeCP2 are provided by the spectrum of mutations causing Rett syndrome and the biochemical and genetic analyses. Recent evidence derived from animal and cellular models suggests that the primary function of this protein is to restrain transcription in a DNA methylation-dependent manner. Interestingly, the resulting defects are reversible in model systems suggesting that the disorder may be curable. Current research addressing these aspects of MeCP2 biology will be presented.

## CONCURRENT SYMPOSIA

### S01 ESHG/EMPAg SYMPOSIUM: The future lies in uncertainty

#### S01.1

##### Tolerating uncertainty in the clinic - the impact of new genetic technologies

*A. Lucassen;*

*University of Southampton, Faculty of Medicine, Southampton, United Kingdom.*

Despite the rhetoric of precision medicine afforded through advances in genetic technologies, and a common perception that genetic tests offer clear cut predictions, the reality in 2016 remains far from this in all but a few distinct areas. Whole genome approaches do provide novel diagnoses or predictions in some families where all previous targeted genetic investigations drew a blank, but for many others the clinical significance of findings from new genetic technologies is unknown until much more evidence -be that bioinformatic or clinical follow up- can be gathered. One of the problems for practice is that the inversion of the phenotype to genotype approach previously used in genetic clinics, to predicting phenotypes from geno[me]types is a quantitative qualitative as well as quantitative leap. This has important implications for how we seek consent for, for example, whole genome sequencing. We can no longer expect patients to be able to have considered all the possible outcomes from such testing in any detail as part of a consent process. Indeed as genomic medicine becomes a mainstream activity, discovery of genomic predispositions to disease that lies outside the clinical experience of the consenter will become more common place. Is 'fully informed' consent a desirable goal in genomic testing? Or should we instead begin to focus on mechanisms that engender greater trust in the venture in question? Using the example of 'incidental' findings from genomic testing, I will explore whether we are placing too much emphasis on consent as a gateway to testing and whether this, perhaps paradoxically, carries the risk of accusations of paternalism. Consent to genomic testing with a full understanding of all the possible outcomes and consequences will not be possible so we will need to consider how best to obtain broad or generic consent to testing that is realistic about the uncertainties and builds in mechanisms for revised interpretation in the wake of new knowledge.

#### S01.2

##### Personal genomic testing: How individuals understand and respond to genetic risk information

*S. Roberts;*

*University of Michigan School of Public Health, Ann Arbor, MI, United States.*

This presentation will discuss how individuals appraise and respond to genetic susceptibility testing for a range of medical conditions. Topics to be addressed include public understanding and attitudes regarding testing options, challenges in communicating test results, psychological effects of genetic risk information, and health behavior changes following testing. The author will draw upon his research program that includes the following projects: 1) the REVEAL (Risk Evaluation and Education for Alzheimer's Disease) Study, a series of randomized clinical trials examining the impact of different methods of disclosing APOE genotype status to individuals at risk for Alzheimer's disease, and 2) the Impact of Personal Genomics (PGen) Study, a longitudinal survey of over 1800 consumers of direct-to-consumer genetic testing services. Implications of research findings for practice and policy will be discussed.

### S02 Understanding functional effects of genomic variants

#### S02.1

##### Using single-cell RNA-seq to understand effects of rare variants

*A. Sekar<sup>1,2</sup>, A. R. Bialas<sup>3,4</sup>, H. de Rivera<sup>1,5</sup>, A. Davis<sup>1,5</sup>, T. R. Hammond<sup>3</sup>, N. Kamitaki<sup>1,5</sup>, K. Tooley<sup>1,5</sup>, J. Presumey<sup>6</sup>, M. Baum<sup>1,5</sup>, V. Van Doren<sup>7</sup>, G. Genovese<sup>1,5</sup>, S. A. Rose<sup>8</sup>, R. E. Handsaker<sup>1,5</sup>, Schizophrenia Working Group of the Psychiatric Genomics Consortium, M. J. Daly<sup>5,6</sup>, M. C. Carroll<sup>1,8</sup>, B. Stevens<sup>5,3</sup>, S. A. McCarroll<sup>1,5</sup>.*

<sup>1</sup>Department of Genetics, Harvard Medical School, Boston, MA, United States, <sup>2</sup>MD-PhD Program, Harvard Medical School, Boston, MA, United States, <sup>3</sup>Department of Neurology, F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical

*School, Boston, MA, United States, <sup>4</sup>Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA, United States, <sup>5</sup>Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>6</sup>Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, United States, <sup>7</sup>Harvard Medical School, Boston, MA, United States.*

Schizophrenia is a heritable brain illness with unknown pathogenic mechanisms. Schizophrenia's strongest genetic association at a population level involves variation in the major histocompatibility complex (MHC) locus, but the genes and molecular mechanisms accounting for this have been challenging to identify. Here we show that this association arises in part from many structurally diverse alleles of the complement component 4 (C4) genes. We found that these alleles generated widely varying levels of C4A and C4B expression in the brain, with each common C4 allele associating with schizophrenia in proportion to its tendency to generate greater expression of C4A. Human C4 protein localized to neuronal synapses, dendrites, axons, and cell bodies. In mice, C4 mediated synapse elimination during postnatal development. These results implicate excessive complement activity in the development of schizophrenia and may help explain the reduced numbers of synapses in the brains of individuals with schizophrenia.

#### S02.2

##### RNA splicing is a primary link between genetic variation and disease

*Y. Gilad;*

*Chicago, IL, United States.*

Noncoding variants play a central role in the genetics of complex traits, but we still lack a full understanding of the molecular pathways through which they act. We quantified the contribution of cis-acting genetic effects at all major stages of gene regulation from chromatin to proteins, in Yoruba lymphoblastoid cell lines (LCLs). About ~65% of eQTLs (expression Quantitative Trait Loci) have primary effects on chromatin, while the remaining eQTLs are enriched in transcribed regions. Using a novel method, we also detected 2,893 splicing QTLs, most of which have little or no effect on gene-level expression. These splicing QTLs are major contributors to complex traits, roughly on a par with variants that affect gene expression levels. Our study provides a comprehensive view of the mechanisms linking genetic variation to variation in human gene regulation.

#### S02.3

##### Modeling genetic and non-genetic sources of variation in single cells

*O. Stegle;*

*Cambridge, United Kingdom.*

Recent technological advances have enabled assaying single-cell transcriptomes and DNA methylation on a genome-wide scale. In this talk, I will discuss some of the computational challenges when analyzing these data from genetics perspective. I will revisit statistical approaches for decomposing the sources of variation between cells using on linear mixed models, which is similar to approaches used for estimating heritability in genetics. These methods enable decomposing single-cell heterogeneity into biological divers, confounding factors and genetic effects. One of the main challenges when linking genetic variation with single-cell states is that most studies profiles cells from very few distinct genetic backgrounds. We show that recent advances in learning and inference can help to overcome these limitations, such that genetic effects on single-cell variability can be elucidated using cells from a single genome.

### S03 Hereditary cancer

#### S03.1

##### Hereditary colorectal cancer: Recent advances and future perspectives

*L. Valle;*

*Catalan Institute of Oncology, IDIBELL, Barcelona, Spain.*

Family history of cancer is one of the strongest predictors of colorectal cancer (CRC) risk, being this risk higher with increasing number of affected relatives and when CRC occurs at young age. Crude estimates indicate that 20-25% of all CRC patients have at least one relative affected with the disease, which may be explained by shared genetic and/or environmental factors. Approximately 3-6% of all CRC patients carry germline mutations associated with syndromic hereditary colorectal cancer. This genetic predisposition to CRC has been classically associated with germline mutations (and epimutations) in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and

PMS2 for non-polyposis cases, and in *MUTYH* and *APC* for adenomatous colonic polyposis. Other CRC predisposing syndromes, characterized by the presence of hamartomatous polyps, are caused by mutations in *SMAD4*, *BP1*, *PR1A*, *STK11* and *PTEN*. Despite this knowledge, much of the genetic predisposition to CRC remains unexplained.

The identification of a germline mutation that causes the increased risk and aggregation of CRC in a family has clear consequences in the clinical management of its members. Therefore, important efforts are being invested to identify novel genes that explain this predisposition, in particular in families with clear dominant inheritance of the disease. In the last decade, the rapid development and decrease in the economic cost of massively parallel sequencing-based approaches and genome-wide copy number techniques has allowed the identification of novel genes responsible of hereditary CRC cases, in some instances also associated with polyposis. For some of the identified genes, the evidence gathered to date is robust and their testing has been included in routine genetic diagnostics, while for others, the identification of additional pathogenic mutations in high risk families is mandatory to provide the required evidence to consider the study of the gene in the clinical setting. I will review the latest advances in this field and discuss current challenges and future perspectives.

### S03.3

#### The genetics of skin and uveal melanoma

N. K. Hayward;

QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Family and twin studies indicate that cutaneous melanoma (CM) susceptibility has a strong genetic component. CM sometimes runs in families in which there is an inherited mutation in a single 'high penetrance' gene, but in the general population CM predisposition is likely governed by variation in a combination of 'low penetrance' genes. To date, genome-wide association studies (GWAS) have identified 20 low penetrance loci associated with CM susceptibility in populations of European descent. The majority of these loci are related to well characterised CM risk phenotypes of light pigmentation and high numbers of melanocytic naevi. They include variants in or near pigmentation genes: *MC1R*, *ASIP*, *TYR*, *SLC45A2*, *OCA2*, *IRF4* and *TYRP1*; and genes associated with naevus count: *MTAP/CDKN2A*, *PLA2G6*, *IRF4* and *TERT/CLPTM1L*. Ten additional melanoma susceptibility loci not associated with either of these risk phenotypes have been identified. These lie in or near *ATM*, *CASP8*, *CCND1*, *MX2*, *FTO*, *PARP1*, *AGR3*, *CDKAL1*, *RAD23B* and *SETDB1*, suggesting a number of other pathways are involved in melanoma susceptibility. The *ATM* and *PARP1* loci allude to involvement of DNA repair, while *CCND1* and *CASP1* point to regulation of cell proliferation or death respectively. High penetrance germline mutations in the *CDKN2A* and *CDK4* genes account for susceptibility in ~40% of case-dense CM families. Recently, exome sequencing has identified several new familial CM genes. Initial success was the finding of a variant (p.E318K) in *MITF*, the lineage specific transcription factor and oncogene for melanoma, which was 2.33 times more prevalent in melanoma cases than population controls, indicating it is a medium-penetrance melanoma susceptibility variant. Subsequently, the identification of rare germline gain-of-function mutations in the promoter of *TERT*, as well as inactivating mutations in genes encoding the shelterin components *POT1*, *ACD* and *TERF2IP*, implicate telomere dysregulation as a novel risk pathway in familial melanoma. Additionally, mutations in *BAP1* have been associated both with CM and uveal melanoma (UM) predisposition, as well as susceptibility to mesothelioma and a range of other cancers. *BAP1* is currently the only documented high penetrance UM gene, however it is responsible for susceptibility in only ~5% of UM families, therefore other as yet unidentified familial UM genes are likely to exist. In contrast to CM, there have been no GWAS for UM to date. A worldwide collaborative effort is needed to establish such an endeavour to increase our understanding of UM genetics.

### S04 From families to populations and back

#### S04.2

#### Population biobanks: a crucial hub in clinical research

C. Wijmenga;

University Medical Center Groningen, Department of Genetics, Groningen, Netherlands.

Next generation genetics builds bridges between clinical research and population biobanks. Many diseases are multifactorial in origin, implying that they are caused by a combination of genetic and environmental components. To date, common genetic variants have been identified associated

with almost every multifactorial disease or trait. Nevertheless the relation between carrying genetic risk factors and disease outcome is not clear-cut as some people develop disease while others are resilient despite carrying many genetic risk alleles. Population-based prospective cohort studies follow individuals before the onset of disease, allowing for studies that can identify biomarkers and disease-modifying effects (including protective factors). The LifeLines Cohort Study is a large population-based cohort study and biobank that was established as a resource for research on complex interactions between environmental, phenotypic and genomic factors in the development of multifactorial diseases. Baseline phenotypes, biochemistry and dietary data have been collected for 167,729 participants, aged 6 months to 93 years. Genetic information by GWAS is available for about 10% of the cohort. From a subset of approx. 1500 individuals (LifeLinesDEEP) more detailed genomic data was collected including genome-wide RNAseq, methylation, metabolomics and gut metagenomics, allowing us to investigate the intricate relationship between host genetics, diet and gut microbiome on the susceptibility to multifactorial diseases. Based on RNAseq data we are able to assess which common genetic risk variants impact the expression of nearby (cis-eQTL) or distant (trans-eQTLs) genes, even in a context-dependent manner by deconvolution of the data into different blood cell types. Allele specific expression (ASE) even allows investigation of single patients from clinical families. A comprehensive analysis of the gut microbiome revealed strong associations with dietary habits, life-style, medication use and health parameters. We showed for example that the use of proton pump inhibitors - one of the most widely used drugs in Western populations - lead to a less healthy gut microbiome. We also showed that the gut microbiome plays a larger role in the variation in body mass index and blood lipid levels than host genetics. Our study supports the potential of microbiota-modifying intervention by diet, prebiotics or probiotics to prevent or treat multifactorial diseases. The comprehensive analysis as we have performed in LifeLinesDEEP exemplifies a systems approach to understand health and disease and is expected to pave the way towards personalized health care.

#### S04.3

#### Cost effective designs with population specific imputation panels in the Sardinian isolate

S. Sanna;

IRGB-CNR, Monserrato, Italy.

Identification of rare and low frequency variants is possible by whole-genome sequencing, but to assure enough statistical power for analyses several thousands of individuals have to be sequenced, at a cost that is still not negligible. An alternative cost-effective approach is to integrate genotyping arrays data for a large number of individuals with a reference set of sequenced genomes, either using public resources or by direct sequencing a subset of the study samples. The benefits of the latter strategy are expected to be higher for homogenous populations, especially in isolates, as there are fewer haplotypes to be tracked down, and also because rare and less frequent alleles may have raised in frequency due to genetic drift or selection. We have generated a Sardinian-specific reference panel for imputation by low-pass whole-genome sequencing of up to 3514 Sardinians, and used this panel to impute a cohort of 6,602 Sardinian individuals genotyped at ~900K SNPs. We observed that efficiency of imputation was remarkably higher when compared to inferences made with existing public resources, and that low frequency and rare variants could be predicted with proper precision. Inferred genotypes were subsequently tested for association with several quantitative traits, revealing that this genotyping-combined with population-specific sequencing approach provides not only an average benefit in accuracy, but also leads to the discovery of novel associations that would have been missed otherwise. Novel associated loci showing strong differences in frequency and linkage disequilibrium patterns compared to populations in 1000 Genomes or at which the causative variant was absent from the publicly available reference panels were indeed misplaced or completely missed when a non-Sardinian source of haplotypes was used for imputation. Overall, these results demonstrate the benefits of a population-specific panel in Sardinians for complex traits genetics.

## S05 Circulating Cell-Free Nucleic Acids

### S05.3

#### Genomewide plasma DNA sequencing for cancer detection and noninvasive prenatal testing

*Y. Lo;*

*Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China.*

Recently, there is a lot of interest on the use of circulating cell-free DNA in plasma for molecular diagnostics. In particular, the success in noninvasive prenatal testing using cell-free fetal DNA in maternal plasma has demonstrated the power of this approach. We have ongoing efforts to explore the applicability of a similar approach for the noninvasive detection of cancer, using genomewide plasma DNA sequencing. Using this approach, we have found that cancer-associated copy number aberrations and methylation aberrations can be detected in a genomewide manner in the plasma of cancer patients. We have also demonstrated that circulating tumor-derived DNA is shorter than that from non-tumor tissue in plasma. Very recently, we have shown that by using over 5800 methylation markers showing tissue-specific methylation changes, one can profile the origin of DNA in plasma. Using this approach, one can pinpoint the tissue of origin of an observed plasma DNA aberration, thus helping one to localize the site of a putative cancer. This technology, called plasma DNA tissue mapping, has also shed light on the origin of circulating DNA in healthy subjects. In this regard, we have shown that most of the circulating DNA in healthy subjects is derived from myeloid and lymphoid cells. We believe that plasma tissue mapping has diagnostic applications in many other clinical scenarios associated with cell death phenomena.

## S06 Behavioural disorders

### S06.1

#### Using next-generation sequencing to understand the genetics of autism

*E. Eichler<sup>1,2</sup>;*

<sup>1</sup>*Seattle, WA, United States, <sup>2</sup>University of Washington & HHMI, Seattle, WA, United States.*

Autism is a devastating neurodevelopmental disorder that afflicts approximately 1% of live births. It is characterized by deficits in language, social interaction and repetitive behaviors. I will summarize our findings regarding the discovery of genetic mutations and their contribution to autism spectrum disorder (ASD) and developmental delay (DD). I will present evidence from exome and molecular inversion probe sequencing of more than 10,000 children with simplex autism and show how these data may be used to pinpoint specific genes. The emerging data strongly argue that the development of the human brain is particularly sensitive to the timing and expression of many different genes; multiple genetic perturbations within specific neurodevelopmental pathways related to long-term potentiation, chromatin remodeling, synaptic function and Wnt signaling appear particularly important; and that the maternal and paternal contributions differ significantly. I will present data on how grouping patients based on a specific gene can be used to predict clinical subtypes of autism. Next-generation exome and genome sequencing data provide a powerful path forward for understanding the genetic architecture of these diseases but the heterogeneity demands an unprecedented level of global cooperation and networking.

### S06.2

#### A highly conserved program of neuronal microexons is misregulated in autistic brains

*M. Irimia<sup>1</sup>, M. Quesnel-Vallieres<sup>2</sup>, N. Parikshak<sup>3</sup>, D. H. Geschwind<sup>3</sup>, S. P. Cordes<sup>4</sup>, B. J. Blencowe<sup>2</sup>;*

<sup>1</sup>*Centre for Genomic Regulation (CRG), Barcelona, Spain, <sup>2</sup>University of Toronto, Toronto, ON, Canada, <sup>3</sup>University of California Los Angeles, Los Angeles, CA, United States,*

<sup>4</sup>*Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada.*

A key challenge in understanding autism is to establish whether this genetically heterogeneous disorder is caused by common mechanisms. Transcriptomic profiling has revealed consistently altered signatures in autistic brains. These include a program of 3-27 nucleotide 'microexons', which is misregulated in the brains of individuals with autism spectrum disorder. Relative to all other types of alternative splicing, microexons display the most striking evolutionary conservation and switch-like regulation during

neuronal differentiation. These microexons modulate the function of interaction domains of proteins involved in neurogenesis and are modulated by neuronal activity. Most neural microexons are regulated by the neuronal-specific splicing factor nSR100/SRRM4 through its binding to adjacent intronic enhancer motifs, and reduced levels of this protein are also observed in autistic brains. Collectively, these results provide evidence that misregulation of a splicing network affected by neuronal activity is associated with autism spectrum disorder.

### S06.3

#### Genetics of schizophrenia

*M. O'Donovan;*

*MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, United Kingdom.*

Risk alleles for schizophrenia span the full spectrum of population frequencies and include common alleles of small effect sizes and rare alleles with large effect sizes. The past few years has seen a rapid expansion in the number of robustly implicated genetic risk factors associated with schizophrenia, increasingly facilitating analyses seeking to link genetic findings to biological processes that underlie some aspects of pathophysiology. In this presentation, I will summarize the progress in identifying schizophrenia associated variants from a diverse set of study designs based on published and unpublished data, show that they appear to converge on proteins involved in various facets of synaptic biology, and highlight work showing that some of the risk alleles have manifestations that are apparent in early childhood.

## S07 Long distance regulation of transcription and translation

### S07.1

#### Gene regulation dynamics and chromatin architecture during development and evolution

*J. L. Gómez-Skarmeta;*

*Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas/Universidad Pablo de Olavide, Sevilla, Spain.*

Temporal and evolutionary dynamic of gene expression is critical for tissue formation during animal development and has been essential for morphological diversification along evolution. This depends on *cis*-regulatory information located at the non-coding DNA. What is the dynamic of this regulatory information along development and evolution and how this information is organized in the genome? Here I will present a comparative study of the vertebrate epigenome during development that reveals an evolutionary conserved process at *cis*-regulatory regions essential for vertebrate body plan formation. In addition, I will show the evolutionary history of the 3D chromatin architecture of critical genomic loci.

### S07.2

#### Dissecting mammalian transcriptional organization by analyzing the content of transcription factories

*A. Papantonis;*

*Cologne, Germany.*

Mammalian cell nuclei contain three RNA polymerases (I, II, and III), each transcribing a different gene subset. The active forms of these polymerases are contained in supra-molecular complexes known as 'transcription factories'. These complexes harbor >95% of nuclear transcriptional activity, and are embedded in the 3D structure of the nucleus. We recently described a novel method by which the protein and RNA contents of transcription factories can be isolated and analyzed in a high throughput fashion. Using these approaches we may now directly interrogate aspects of mammalian transcriptional organization that remain elusive. For example, using nascent RNA isolated from factories, we were able to show for the first time that many human introns are spliced in a recursive manner. This molecular mechanism utilizes cryptic, non-canonical, sites embedded in introns. The tight association of these sites with intragenic *cis*-regulatory elements, and potentially with underlying genetic variants, suggest a whole new way by which results from both genetic and functional studies should now be approached.

## S08 Sensory disorders

### S08.1

#### Functional studies reveal how mutations of MYO15A cause human deafness DFNB3

T. B. Friedman, A. U. Rehman, J. E. Bird;

National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, MD, United States.

Autosomal recessive mutations in any one of almost a hundred genes account for nearly half of the irreversible non-syndromic deafness found in newborn humans. Additionally, there are at least 400 clinically distinct syndromes described in the Online Mendelian Inheritance in Man that involve hearing loss. Twenty years ago, deafness segregating in a Balinese village was mapped to chromosome 17p11.2 and designated the DFNB3 locus. Subsequently, missense mutations of myosin 15 were found to cause DFNB3 and the shaker 2 deaf mouse phenotype. Across diverse ethnic groups, recessive mutations of MYO15A encoding myosin 15 are now recognized as a common cause of congenital, profound deafness. Myosins are molecular motors that generate tension and produce force to power motility along cytoskeletal actin filaments. Three distinct alternative splice isoform classes of myosin 15 have been discovered, of which the 395 kDa (3,530 residues) isoform 1 is the largest member of the myosin superfamily. Presently, 192 different recessive mutations of MYO15A associated with DFNB3 are distributed across 49 of its 67 exons (43% missense, 23% frameshift, 16% nonsense, 16% splice site and 2% indels). Several of the truncating mutations of MYO15A are located in giant exon 2 which alone encodes 1,233 amino acid residues, and yet it is surprising that there isn't a single convincing pathogenic missense mutation reported in this exon. The pathogenicity of MYO15A mutations and the wild-type functions of myosin 15 have been gleaned from biochemical studies and animal models of DFNB3. Isoform 2 of myosin 15 transports whirlin and EPS8 to the tips of stereocilia. This complex is essential for the metamorphosis of microvilli into mechanosensitive stereocilia bundles on the apical surface of hair cells. Shortly after birth, hair cells then switch expression to myosin 15 isoform 1 that maintains adult actin-cytoskeleton of stereocilia and is necessary for life-long hearing. Supported by NIDCD/NIH Intramural funds DC000048-19 to TBF

### S08.2

#### Genetics of visual impairment

D. Schorderet;

Institut de Recherche en Ophtalmologie, Sion, Switzerland.

Inherited diseases of the eye encompass a large number of entities including recessive and dominant diseases, autosomal, X-linked and mitochondrial. As the eye is a complex structure, diseases affecting each of its structures may induce loss of vision. Developmental anomalies, corneal dystrophies, glaucoma, rod-cone or cone-rod dystrophies, vitreoretinopathies and even cancer may all lead to visual impairment. During this presentation I will focus on diseases of the cornea and retina, describing some of the most prevalent disorders. I will also show how current genetic research has accelerated the pace of discovery of new genes and discuss why geneticists should interplay much more with ophthalmologists for a better management of patients.

## S09 ASHG/ESHG/EMPG SYMPOSIUM: Debating germline genome editing

### S09.3

#### Ethical aspect of germline gene editing

A. Bredenord;

Utrecht, Netherlands.

Since the advent of recombinant DNA technology in the 1970s, genetic testing, selection and screening have been considered to be ethically justifiable and allowed under most jurisdictions, but genetic modification of the human germline has for many been an ethical line that should not be crossed. Germline modification encompasses any biomedical intervention that modifies the genome that a person can transmit to his or her child and the child's entire lineage. Until recently, the technological tools that enable a viable path to realizing human germline modification were not available. However, two recent scientific developments have revived the international discussion on the ethics of germline modification and underscore the need to address the ethics and governance of human germline modification now.

First, UK parliament recently legalized a novel reproductive genetic technology to prevent mitochondrial DNA disorders: mitochondrial replacement techniques. Now both the technological barriers as well as the policy barriers for MRT are considerably lowered, at least in the UK, the first clinical use is expected shortly. This would encompass a modification of the mitochondrial genome. Second, recent advances in genome editing techniques such as CRISPR/Cas9 make it possible to edit DNA sequences in virtually any organism of choice. In April 2015, Chinese scientists published the first report of the use of gene editing techniques for basic research in human embryos. Gene editing experiments in human embryos have now been approved in China, the UK and Sweden. These developments have sparked an international ethical debate. Leading scientific journals *Nature* and *Science* rejected the first Chinese publication for ethical reasons and published commentaries that called for an international moratorium. Others view the continuation of basic gene editing research on human embryos as a 'moral imperative' for its offers the future potential of correcting genetic disease, many of which are currently untreatable. Many, among which myself as a member of the Hinxton Group, emphasized the need for ethical and public deliberation about emerging genome editing techniques and (international) oversight. In this presentation I will review the key ethical arguments in favor and against germline modification and identify some of the ethical and governance questions that need to be examined in the coming period.

### S09.4

#### ASHG statement on germline genome editing

K. Ormond;

Stanford, CA, United States.

The American Society of Human Genetics (ASHG) developed a workgroup to create a policy statement on human germline genome editing. This workgroup consists of a combination of clinician scientists and biomedical ethicists from the United States, Canada, and the UK. Somatic gene editing applications fall under already established guidelines in most countries, and are therefore not included in this statement. The presentation will discuss the current status of the policy draft and our key positions, as well as the ethical justifications.

## S10 Brain genetics

### S10.1

#### The genetics of brain calcification

C. Klein<sup>1,2</sup>;

<sup>1</sup>Lübeck, Germany, <sup>2</sup>Institute of Neurogenetics, Lübeck, Germany.

Linkage analyses of large families affected by autosomal dominant primary familial brain calcification (PFBC; formerly known as idiopathic basal ganglia calcification (IBGC)) identified three candidate loci (named IBGC1-3). In 2012, mutations in SLC20A2 were found within the IBGC3 locus. In the following years, using next-generation sequencing, three further PFBC genes (PDGFRB, PDGFB, XPR1) were discovered and the respective disease forms were subsequently designated as IBGC4-6. A systematic search of the MEDLINE database for articles published until 2014 for the three genes with more than one report in the literature (SLC2012, PDGFRB, and PDGFB) and a search of individual reference lists of all identified articles left 15 reports of genetically confirmed cases with individual clinical information. A total of 179 cases were reported, including 162 individuals who belonged to 25 families. Availability of information ranged from 96.6% for ethnicity to 24.4% for age at onset. All cases, regardless of which of the three genes was mutated, had calcifications on comprehensive cranial computed tomography, most frequently located in the basal ganglia (70.6%), subcortical white matter (40.8%), cerebellum (34.1%), or thalamus (28.5%). Mean (SD) AAO was 27.9 (22.3) years, and the AAO was likewise comparable across genes ( $P = .77$ ). The most frequently described clinical signs were movement disorders, such as parkinsonism (12%) and dystonia (19%). Penetrance of the imaging phenotype was 100% compared with only 61% of the clinical phenotype. Pathophysiological pathways of all four genes involved in PFBC converge on impaired phosphorus homeostasis and integrity of the blood-brain barrier.

## S11 Human genomics: transcriptome, proteome and interactome

### S11.1

#### The human transcriptome across tissues and individuals

R. Guigó;

Center for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, Spain.

The pilot phase of the Genotype-Tissue Expression (GTEx) project has produced RNASeq from 1,641 samples originated from up to 43 tissues from 175 post-mortem donors, and constitutes a unique resource to investigate the human transcriptome across tissues and individuals. Clustering of samples based on gene expression recapitulates tissue types, separating solid from not solid tissues, while clustering based on splicing separates neural from non-neural tissues, suggesting that post-transcriptional regulation plays a comparatively important role in the definition of neural tissues. About 47 % of the variation in gene expression can be explained by variation of across tissues, while only 4% by variation across individuals. We find that the relative contribution of individual variation is similar for lncRNAs and for protein coding genes. However, we find that genes that vary with ethnicity are enriched in lncRNAs, whereas genes that vary with age are mostly protein coding. Among genes that vary with gender, we find novel candidates both to participate and to escape X-inactivation. In addition, by merging information on GWAS we are able to identify specific candidate genes that may explain differences in susceptibility to cardiovascular diseases between males and females and different ethnic groups. We find that genes that decrease with age are involved in neurodegenerative diseases such as Parkinson and Alzheimer and identify novel candidates that could be involved in these diseases. In contrast to gene expression, splicing varies similarly among tissues and individuals, and exhibits a larger proportion of residual unexplained variance. This may reflect that that stochastic, non-functional fluctuations of the relative abundances of splice isoforms may be more common than random fluctuations of gene expression. By comparing the variation of the abundance of individual isoforms across all GTEx samples, we find that a large fraction of this variation between tissues (84%) can be simply explained by variation in bulk gene expression, with splicing variation contributing comparatively little. This strongly suggests that regulation at the primary transcription level is the main driver of tissue specificity. Although blood is the most transcriptionally distinct of the surveyed tissues, RNA levels monitored in blood may retain clinically relevant information that can be used to help assess medical or biological conditions.

### S11.3

#### Mapping Human Long Noncoding RNAs

R. Johnson;

Barcelona, Spain.

The Gencode manually-curated gene annotation is a fundamental resource for long noncoding RNA (lncRNA) research. Despite rapid growth it remains incomplete, both in terms of missing gene structure elements (splice sites, exons, transcription start and termination sites) in annotated genes, as well as unknown numbers of unannotated genes.

To address both issues, we are applying RNA Capture methodology coupled to Third Generation long-read sequencing ("Capture Long-Seq", CLS) in both Human and Mouse. We have designed capture libraries that target (a) the entire set of known intergenic lncRNA genes, and (b) thousands of regions suspected to harbour unannotated lncRNA based on small RNA, enhancer, conservation or orthology evidence.

Analysis of > 2 million complete long reads of captured RNA from 6 tissues deepens known lncRNA annotations several times over: using conservative filters, the set of canonical lncRNA splice junctions increases by 250%. Around 80% of lncRNAs are detected, at a mean depth of 70 reads. We observe numerous cases of new exons, unified gene models underlying multiple annotations, and mono-exonic genes. Taking advantage of long read information, we also map 132,305 polyadenylation sites genome-wide.

Combining this data with CAGE transcription start site maps, we create the first annotation of confident, complete lncRNA transcript models. We use this to define the fundamental properties of lncRNA gene structure.

## S12 SeX and the citY

### S12.1

#### Sex determination: why so many ways of doing it?

D. Bachtrog;

Tree of Sex Consortium, Berkeley, CA, United States.

Sexual reproduction is an ancient feature of life on earth, and the familiar X and Y chromosomes in humans and other model species have led to the impression that sex determination mechanisms are old and conserved. In fact, males and females are determined by diverse mechanisms that evolve rapidly in many taxa. Yet this diversity in primary sex-determining signals is coupled with conserved molecular pathways that trigger male or female development. Conflicting selection on different parts of the genome and on the two sexes may drive many of these transitions, but few systems with rapid turnover of sex determination mechanisms have been rigorously studied. I will discuss our current understanding of how and why sex determination evolves in animals and plants and identify important gaps in our knowledge that present exciting research opportunities to characterize the evolutionary forces and molecular pathways underlying the evolution of sex determination.

### S12.2

#### Origins and functional evolution of Y chromosomes across mammals

H. Kaessmann;

Heidelberg University, Heidelberg, Germany.

Y chromosomes underlie sex determination in mammals, but their repeat-rich nature has hampered sequencing and associated evolutionary studies. In an ongoing project, we are tracing Y functional evolution across representative mammals based on various types of high-throughput sequencing data. We previously uncovered three independent sex chromosome originations in mammals and birds (the outgroup). The original placental and marsupial (therian) Y, containing the sex-determining gene SRY, emerged in the therian ancestor approximately 180 million years ago, in parallel with the first of five monotreme Y chromosomes, carrying the probable sex-determining gene AMH. The avian W chromosome arose approximately 140 million years ago in the bird ancestor. The small Y/W gene repertoires, enriched in regulatory functions, were rapidly defined following stratification (recombination arrest) and erosion events and have remained considerably stable. Despite expression decreases in therians, Y/W genes show notable conservation of proto-sex chromosome expression patterns, although various Y genes evolved testis-specificities through differential regulatory decay. Thus, although some genes evolved novel functions through spatial/temporal expression shifts, most Y genes probably endured, at least initially, because of dosage constraints. In my talk, I will describe these findings as well as other interesting aspects of Y chromosome evolution that we recently uncovered.

### S12.3

#### A Y-like social chromosome causes alternative colony organization in fire ants

L. Keller;

Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland.

Intraspecific variability in social organization is common, yet the underlying causes are rarely known. In this talk I will show that the existence of two divergent forms of social organization is under the control of a pair of heteromorphic chromosomes that have many of the key properties of sex chromosomes. The two variants, hereafter referred to as the social B and social b (SB and Sb) chromosomes, are characterized by a large region of approximately 13 megabases (55% of the chromosome) in which recombination is completely suppressed between SB and Sb. The lack of recombination can be explained by at least one large inversion, and this absence of recombination has led to the accumulation of deleterious mutations, including repetitive elements in the non-recombining region of Sb compared with the homologous region of SB. Importantly, most of the genes with demonstrated expression differences between individuals of the two social forms reside in the non-recombining region. These findings highlight how genomic rearrangements can maintain divergent adaptive social phenotypes involving many genes acting together by locally limiting recombination. <!--EndFragment-->

## S13 Epigenetic reprogramming in disease

### S13.1

#### Epigenetics in health and disease

*M. Esteller;*

*IDIBELL, Cancer Epigenetics and Biology Program (PEBC), Hospital Duran i Reynals, Barcelona, Spain.*

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasis in neoplasia, but without forgetting the novel advances in other human disorders such as neurodegenerative or cardiovascular diseases. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs. Beyond that, epigenetics is involved in the aging process and many others aspects of human biology.

### S13.2

#### Intergenerational and Stochastic Epigenetic Control of Metabolic Disease: Evidence for polyphenism in mouse and man?

*J. A. Pospisil;*

*Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany.*

More than one half billion people are obese. Though substantial progress is being made in understanding genetic predisposition for obesity, much of the high heritability for the disease remains enigmatic. Our lab focuses on understanding how chromatin state biases can be established to generate or modify disease susceptibility. We have begun to dissect how core chromatin regulatory systems can impact metabolic phenotypes, their variability and their distribution. In a first study, we have identified a paradigm of paternal-diet-induced intergenerational metabolic reprogramming (IGMR). We find that diet can act as a physiological suppressor of variegation, capable of selectively modifying genomic output for the lifetime of the offspring individuals. This occurs in a chromatin-state-specified manner and controls transcriptome signatures in mature sperm of the father and in offspring embryos. This model has allowed us to identify Polycomb and core-heterochromatin machinery as required for the manifestation of intergenerational metabolic reprogramming (Ost, Cell 2014). In a second study, we identify a Trim28-sensitive epigenetic switch that triggers obesity stochastically and in an apparently 'On/Off' manner. Using genetic dissection and transcriptome analyses, we map this phenotype to control of a series of metabolically potent imprinted genes. We find that human children present in similarly distinct sub-populations stratifying by *Trim28* expression, imprinted gene dysregulation, and profoundly distinct transcriptome organization (Dalgard Cell 2016). To the best of our knowledge, the latter data provide the first evidence of *polyphenism* in mouse and humans and break major assumptions made in our understanding of complex trait genetics, evolution and medicine. In the presentation, I will provide our latest updates on these ideas as well as their extensions towards tissue specificity of function.

### S13.3

#### Epigenetic reprogramming in the germline and early embryo

*G. Kelsey, K. Stewart, L. Veselovska, S. Smallwood, C. Hanna, J. Huang, H. Saadeh; The Babraham Institute, Cambridge, United Kingdom.*

Epigenetic mechanisms are fundamental for the orderly regulation of gene expression throughout the life-course, providing a critical memory of ear-

lier decisions. They can ensure resilience against environmental change, but also allow adaptation. Nowhere is this more important than in the gamete and the early embryo. We are seeking to understand what governs how DNA methylation and other epigenetic marks are patterned during gametogenesis and embryogenesis. Using mouse as a model, we have shown that transcription is the major determinant of the DNA methylation landscape in the oocyte. To understand the mechanistic connection between transcription and de novo methylation, we have profiled histone modifications in growing oocytes and identify chromatin states permissive for and resistant to DNA methylation characterised by reciprocal enrichment of H3K4me2/me3 and H3K36me3. This finding is reinforced by the consequences on the DNA methylation landscape of genetically ablating specific H3K4 demethylases and methyltransferases. Key to such studies is the development of methods for genome-wide profiling of epigenetic marks in low numbers of cells, including at the single-cell level, which could have application widely in epigenetic studies, particularly for rare cell types and for investigating cell-to-cell heterogeneity in epigenetic marks at critical developmental transitions.

## S14 Reproductive genetics and genomics

### S14.1

#### Cell fate decisions during early development

*M. Zernicka-Goetz;*

*University of Cambridge, Dept. of Physiology, Development & Neuroscience, Cambridge, United Kingdom.*

Embryonic development progresses through successive cell fate decisions and intricate three-dimensional morphogenetic transformations. Implantation is the defining event in mammalian pregnancy during which a fundamental morphogenetic transformation is initiated: the body axes are established and the embryonic germ layers created. Despite its importance, a comprehensive understanding of the molecular mechanisms, transcriptional pathways, cellular interactions, as well as the spatio-temporal development of the embryo at implantation stages is at present lacking, due to the embryo's inaccessibility. To overcome these limitations, we have generated a culture system that allows the development of implanting embryos outside of the mother. This system provides the opportunity to address how architectural features and signaling events integrate to induce the emergence of the body plan. I will describe our progress in using this system to analyse the pathways underlying the very first morphogenetic changes that occur in the embryo following implantation and how we can mimic these events using ESCs both for the mouse and for human embryos. Our next challenge is to establish how the pluripotent epiblast cells interact with flanking extra-embryonic tissues during this developmental stage to generate AP axis within the embryo and progress to gastrulation.

### S14.2

#### Human Embryo Genome Activation

*J. Kere, V. Tööhönen;*

*Karolinska Institutet, Stockholm, Sweden.*

After fertilization, massive oocyte RNA degradation takes place, followed by a cascade-like activation of transcription from the embryo genome (Embryo Genome Activation, EGA). We have characterized human EGA by single-cell RNA sequencing of over 340 oocytes, zygotes and isolated blastomeres from 4-cell and 8-cell stage embryos, focusing on the transcription start sites (TSS) using the STRT method (Tööhönen, Katayama & al. Nat Comm 6:8207, 2015). Our results revealed that 32 transcripts, including seven previously incompletely annotated PRD-like homeodomain transcription factor genes, were the first to be transcribed at 4-cell stage (day 2 after fertilization), followed by 129 transcripts at 8-cell stage (day 3). The total content of mRNA remained essentially unchanged in oocytes and zygotes, but was reduced 16-fold in 4-cell blastomeres, consistent with the cell division effect and oocyte transcript degradation. Supporting degradation, at 4-cell stage the majority of transcript reads mapped elsewhere than the 5' promoter and upstream regions of genes. For the first activated genes, our TSS targeted data allowed the identification of critical regulators of EGA as 36 bp and 35 bp conserved promoter elements at the two stages of EGA, respectively. We cloned and verified the genomic structures of seven homeobox genes not present in the rodent genomes: ARGFX, CPHX1, CPHX2, DPRX, DUXA, DUXB, and LEUTX. Exploration of FANTOM5 data and other databases showed exclusive expression of these genes during the EGA and absent expression already in human embryonal stem cells (hESCs). Our functional analyses confirm their activity as transcription factors with a spectrum of activity

from strong activators to strong suppressors of transcription in hESC models (Jouhilahti, Madissoon & al. unpublished work). Our data constitute a resource for understanding the earliest steps of human embryonal development and provide new genes of interest for the study of pluripotency and stem cell technologies.

#### S14.3

#### Mechanisms of lineage specification in human embryos and stem cells

**K. Niakan;**

*The Francis Crick Institute, London, United Kingdom.*

During early human development totipotent zygotes diverge into pluripotent embryonic cells, which form the fetus, and extra-embryonic cells, which contribute to the placenta and yolk sac. Understanding the molecular mechanisms that regulate pluripotency in human embryos and how it is disengaged during cellular differentiation is of fundamental biological importance. Using single-cell RNA-sequencing of human and mouse embryos we have elucidated conserved transcriptional programs along with those that are human-specific. By modulating signaling pathways we discovered a requirement for TGF- $\beta$  signaling in the maintenance pluripotency in human embryos. By uncovering the molecular basis of these early cell lineage decisions we underscore their significant clinical implications for infertility, miscarriages, developmental disorders and therapeutic applications of stem cells.

### S15 Therapy in rare diseases

#### S15.1

#### Gene therapy in Fanconi anemia

**J. A. Bueren;**

*Division of Hematopoietic Innovative Therapies. CIEMAT, CIBER of Rare Diseases and IIS-Fundación Jiménez Díaz, Madrid, Spain.*

Fanconi anemia (FA) is an inherited disease mainly characterized by congenital abnormalities, progressive bone marrow failure, and cancer predisposition. Gene therapy may constitute a good alternative for the treatment of Fanconi anemia (FA) patients lacking a HLA matched donor. However, in contrast to other disorders already treated by gene therapy, marked proliferation and differentiation defects have been observed in FA hematopoietic stem cells (HSC), complicating the harvesting of a high number of CD34+ cells to be used in gene therapy protocols. On the other hand, the proliferation advantage of gene-corrected FA HSCs may facilitate the hematopoietic reconstitution of the patient by a low number of transduced HSCs. For the collection of HSCs from FA patients, filgrastim and plerixafor have been used as mobilizing agents. The short transduction of small aliquots of mPB CD34+ samples from these patients with a GMP-produced lentiviral vector that harbors the *FANCA* therapeutic gene corrected the phenotype of 20-40% of these progenitor cells. To assess the repopulating ability of transduced FA-A CD34+ cells, samples were transplanted into immunodeficient NSG mice. Remarkably, most of the transplanted samples engrafted the NSG mice, and an *in vivo* proliferation advantage of gene-corrected CD34+ FA-A cells was observed in recipients' BM. Based on these preclinical studies, a gene therapy trial of FA-A patients is currently open in Spain. In addition to conventional gene therapy, we have also investigated the possibility of generating gene-corrected FA HSCs by conducting untargeted and also targeted gene addition approaches on FA fibroblasts that were subsequently reprogrammed to generate induced pluripotent stem cells. An update of our preclinical and clinical studies will be presented.

#### S15.2

#### Antisense therapy in Spinal Muscular Atrophy

**R. Finkel;**

*Orlando, FL, United States.*

Classic spinal muscular atrophy (SMA), a progressive motor neuron disorder, is the most common fatal autosomal recessive disease of infancy, with an incidence of approximately 1:10,000 and carrier frequency of 1:40 to 1:60. SMA is a monogenic disorder due to homozygous deletion or mutation in the survival of motor neuron 1 (*SMN1*) gene at 5q13. An inverted duplication of a nearly homologous "backup" gene, *SMN2*, is present only in humans. *SMN2* differs functionally from *SMN1* due to a c.840C>T substitution which creates an exon splice inhibitor and results in alternative splicing that largely excludes exon 7 and generates a non-functional SMN protein. Approximately 10% of transcripts, however, are full-length and translate a

normal protein. In effect, *SMN2* incompletely rescues an otherwise lethal condition. The number of copies of *SMN2* is inversely related to the severity of the phenotype. There is a broad range in phenotype. Over half of affected patients have the most severe form, type I, which presents in early infancy. These infants fail to sit or gain motor skills and generally die by 2 years of age, due to related respiratory muscle weakness and pulmonary infection. The less severe forms present as: type II, in later infancy with sitting achieved, and type III, in childhood with walking achieved.

Multiple treatment strategies for spinal muscular atrophy have entered clinical trials in recent years. Pre-clinical cell-based and animal model studies have identified several approaches to rescue the phenotype and possibly intervene pre-symptomatically. Initial drug trials in SMA examined repurposed use of existing drugs: HDAC inhibitors to increase gene transcription (valproic acid, phenylbutyrate), modulators of exon 7 inclusion in the *SMN2* gene (valproic acid, hydroxyurea, quinazolones), and inhibitors of excitotoxicity (glutaminergic: riluzole; GABAergic: gabapentin) or muscle mitochondrial enhancement (l-carnitine). None of these demonstrated meaningful clinical benefit.

Current drug development for SMA focuses on three strategies: (1) splice site modulation to promote inclusion of exon 7 in the *SMN2* transcript via: (a) intrathecal delivery of anti-sense oligonucleotides (ASO), "nusinersen" (Ionis/Biogen) or (b) small-molecule oral drugs, "LMI070" (Novartis) and "RG7916" (Roche); (2) intravenous gene transfer therapy using scAAV9-SMN1 (AveXis/Nationwide Children's Hospital) and; (3) down-stream modification via: (a) neuroprotective/mitochondrial enhancement, "olesoxime" (Trophos/Roche), or (b) troponin activation in muscle, "CK-2127107" (Cytokinetics).

This talk will focus mainly upon the ASO approach to generate full-length transcript in target motor neurons, and will summarize the clinical trials that have demonstrated promising improvement in survival and motor function. A pre-symptomatic study is also underway.

**Summary:** Both targeted *SMN2* gene editing drugs and AAV vector mediated *SMN1* gene replacement studies are advancing through clinical trials and show promising early safety and efficacy results. Downstream disease modifying drugs, by targeting motor neurons or muscle, may provide additional supplemental benefit. Combined treatment may eventually offer a rational multidimensional approach to optimizing therapeutic benefit. How early to treat and how much of the phenotype can be rescued remain critical questions.

#### S15.3

#### Novel Approaches to the Treatment of Cystic Fibrosis

**M. D. Amaral;**

*University of Lisboa, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Lisboa, Portugal.*

Cystic fibrosis (CF) is a major life-shortening genetic disease leading to severe respiratory symptoms caused by mutations in CF transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel expressed at the apical membrane of epithelial cells. Absence of functional CFTR from the surface of respiratory cells reduces mucociliary clearance, promoting airways obstruction, chronic infections and ultimately lung failure [1]. To date ~2,000 CFTR mutations were reported [2] but one single mutation - F508del - occurring in ~85% of CF patients worldwide, is associated with intracellular CFTR protein retention and a severe clinical phenotype.

Major clinical advances in treating CF symptoms (with mucolytics, antibiotics, etc) have significantly increased survival beyond the second decade (~25 years in Europe). However, to further increase CF patients life expectancy, CF needs to be treated beyond its symptoms i.e., through treatments addressing the basic defect associated with each CFTR gene mutation [3,4]. One new drug, potentiator VX-770 (ivacaftor/Kalydeco) has hit the clinical setting but only for ~5% of all CF patients, i.e., those bearing G551D and 8 other mutations causing a similar defect in the channel [5]. More recently, a new drug (Orkambi), which combines corrector VX-809 (lumacaftor) rescuing F508del-CFTR to the cell surface with potentiator ivacaftor, went into the clinic, following proven efficacy, albeit modest, in a phase III clinical trial for F508del/ F508del patients [6].

As these therapies correcting defective CFTR become available, we should quickly pre-assess how other CFTR mutations respond to such new drugs. This is the way forward to extend them more CF patients, namely to those with ultra-rare ("orphan") mutations in an effective and expedite way. Indeed, for such mutations, "classical" clinical trials are not possible due to low numbers of patients and their geographic dispersion. It is thus crucial to use the above novel methods to pre-assess directly on patient's cells/tissues how each individual responds to these novel drugs. These can include a swelling assay in intestinal organoids [7] or measurement of CFTR Cl- cur-

rents in polarized primary cultures of nasal cells [8]. Such pre-assessment may become a standard basis for a drug clinical use in a precision medicine approach.

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## S16 Cardiovascular genetics (Joint with ESC)

### S16.1

#### Update on the genetics of thoracic aortic aneurysms

*B. Loey;*

*University of Antwerp/Antwerp University Hospital, Antwerp, Belgium.*

Thoracic aortic aneurysms (TAA) are often asymptomatic but predispose to aortic dissections, which are associated with high mortality rates. Thoracic aortic aneurysms and dissections (TAAD) can be subdivided into syndromic (associated with systemic manifestations) and non-syndromic forms, although recent clinical observations are blurring this distinction. A positive family history occurs in circa 20% of all TAA individuals. Most commonly, familial TAADs segregate in an autosomal dominant manner, but rare autosomal recessive and X-linked families have been described. Non-syndromic forms of TAA are strongly associated with mutations in genes encoding for proteins of the vascular smooth muscle contractile apparatus (ACTA2, MYH11) or its modifiers (PRKG1, MYLK, FOXE3). Syndromic presentations can be linked to alterations in the extracellular matrix (FBN1, FBLN4, MFAP5, LOX) and the transforming growth factor beta signalling pathway (TGFBR1/2, SMAD2/3, TGFBR2/3). Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder in which affected individuals present with ocular, skeletal and cutaneous signs besides aneurysm and dissection. Loeys-Dietz syndrome (LDS) is an aneurysmal connective tissue disorder that can be distinguished from MFS by the unique presence of craniofacial, skeletal, cutaneous, and/or vascular manifestations, prominently including hypertelorism, cleft palate or bifid uvula and arterial tortuosity with aneurysms distant from the aortic root. In addition, aneurysms in individuals with LDS tend to dissect at an earlier age and smaller diameter compared to individuals with MFS. Whereas MFS is caused by mutations in FBN1, coding for an extracellular matrix protein, LDS is caused by loss-of-function mutations in genes coding for components of the transforming growth factor beta signalling pathway. Significant clinical overlap exists between the phenotypes caused by mutations in the currently known LDS genes (TGFBR1/2, SMAD2/3, TGFBR2/3). Recent work has demonstrated that both MFS and LDS lead to dysregulation of the TGFbeta signalling pathway. The latter has opened interesting avenues for the application of new therapeutic strategies. Current next generation sequencing based gene panel testing for TAAD individuals yields a molecular diagnosis in up to one third of the patients, suggesting that several genes remain to be discovered.

### S16.3

#### Molecular mechanisms responsible for inherited hypertension with brachydactyly

*S. Bähring<sup>1</sup>, P. G. Maass<sup>2,3</sup>, A. Aydin<sup>2</sup>, C. Schächterle<sup>2</sup>, O. Toka<sup>4</sup>, E. Klüssmann<sup>2</sup>, F. C. Luft<sup>1,2</sup>,  
<sup>1</sup>Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany, <sup>2</sup>Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany, <sup>3</sup>Harvard University, Cambridge, MA, United States, <sup>4</sup>Department of Pediatric Cardiology, Children's Hospital, Friedrich Alexander University Erlangen, Erlangen, Germany.*

Autosomal-dominant hypertension with brachydactyly (HTNB) is a salt-independent Mendelian syndrome caused by activating mutations in the gene encoding phosphodiesterase 3A (PDE3A). These mutations increase the protein kinase A (PKA)-mediated phosphorylation of PDE3A resulting in enhanced cAMP-hydrolytic affinity and accelerated cell proliferation. The phosphorylated vasodilator-stimulated phosphoprotein (VASP) is dimi-

nished, and parathyroid hormone-related peptide (PTHrP) is dysregulated, potentially accounting for all phenotypic features. Untreated patients die prematurely of stroke; however, hypertension-induced target-organ damage is otherwise hardly apparent. Large-vessel and cardiac function is preserved in HTNB. The patients have normal platelet function. Cell-based studies demonstrated that available PDE3A inhibitors inhibit the mutant isoforms. However, increasing cGMP to indirectly inhibit the enzyme appears to have particular utility. Since our report we have been referred additional families with suspected HTNB. We identified a novel aspartic acid substitution at the same site featured by one of our earlier families (p.G449D). We verified increased PDE3A phosphorylation at Ser438 with a peptide SPOT assay. Our results shed light on PDE3A activation and could be relevant to the treatment of hypertension in the general population.

## EDUCATIONAL SESSIONS

### E1 Novel genome sequencing technologies

#### E1.1

##### Long-read sequencing of complex genomes

*E. Eichler;*

*Department of Genome Sciences and Howard Hughes Medical Institute, University of Washington, Seattle, WA, United States.*

The human genome is arguably the most-well assembled reference assembly yet many gaps remain and aspects of its structural variation remain poorly understood ten years after its finishing. The discovery and resolution of this variation is critical to understanding disease. I will present our most recent work sequencing human and non-human primate genomes using single molecule sequencing (SMS) technology. We have developed methods to detect indel and structural variants from several bases up to 50 kbp. We have closed or extended ~50% of the remaining interstitial gaps in the human genome and find that 80% of these carry long polypyrimidine/purine tracts multiple kilobases in length. Comparing the single haplotype to the human reference we resolve >35,000 structural variants and >500,000 indels at the basepair level with 99.9% sequence accuracy. 92% of insertions and 60% of deletions between 50-5000 bp in length are novel representing large swaths > 15 Mbp of undiscovered genetic variation within human genomes. We find that such sequences vary extensively in copy number and affect functional elements in the genome. In addition, the analysis uncovers other categories of complex variation that have been difficult to assess including mobile element insertions (eg. SVA) as well as inversions mapping within more complex and GC-rich regions of the genome. Our results suggest a systematic bias against longer and more complex repetitive DNA that can now be partially resolved. I will discuss the potential of this technology to create accurate *de novo* assemblies of additional human genomes and non-human that more comprehensively capture the full spectrum of human genetic diversity and its importance to our understanding of genetic variation and disease.

### E2 Genetic Privacy and Data Sharing joint with EMPAG

#### E2.1

##### The hitchhiker's guide to genome hacking

*Y. Erlich;*

*Columbia University, New York, NY, United States.*

We have entered to the era of ubiquitous genetic information for research, clinical care, and personal curiosity. Sharing these datasets is vital to realize the promise of the genetic revolution. However, one growing concern is the ability to protect the genetic privacy of the data originators. Here, I will technically map threats to genetic privacy and emphasize the limitation of mitigation strategies for privacy-preserving dissemination of genetic data. As an alternative to the zero-sum game of privacy versus utility, I will propose to focus on trust-enabling techniques to create partnerships between researchers and participants.

## E2.2

### The role of policy in navigating the privacy landscape and promoting responsible genomic data sharing

*E. S. Dove;*

*University of Edinburgh, Edinburgh, United Kingdom.*

The world is more connected than ever as we craft far-reaching networks of communication. A tangible effect of our networked world in the genomic and personalized medicine context is the increased sharing of genomic and clinical data between professionals, laboratories, hospitals, organizations, patients, participants, and—increasingly—across national borders. Sharing data facilitates both translational and precision medicine, not to mention research discovery. As various stakeholders in biomedical research and clinical practice laudably move towards a data sharing culture, challenges arise. In particular, privacy and data privacy regulation play a large role in defining the proper contours of global data sharing. Data sharing is a social activity, and privacy is fundamentally a social consideration. Like any partnership, there are responsibilities on both sides. A central concern is that genomic (and clinical) data must be collected, used, and shared in ways that respect the fundamental rights and interests of individuals and society, as reflected in national and international laws, and most notably, privacy. Data sharing carries a spectrum of risks, particularly re-identification of individuals and related others, and thereby represents a *prima facie* threat to their privacy. In this presentation, I outline the practices and benefits of genomic data sharing; the dimensions of privacy and data privacy regulation that challenge data sharing; and the ways in which one organization that I am familiar with, the Global Alliance for Genomics and Health, is addressing these challenges.

## E4 Peroxisomal disorders -still a need for metabolic assays?

### E4.1

#### Overview of peroxisomal disorders - diagnosis and management

*N. Braverman;*

*McGill, Montreal, QC, Canada.*

The peroxisome biogenesis disorders (PBD) include the Zellweger spectrum disorders (ZSD) and Rhizomelic chondrodysplasia punctata type 1 (RCDP1). These disorders are due to defects in anyone of 14 PEX genes, whose protein products, or peroxins, are required for the assembly of functional peroxisomes, which includes the process of matrix enzyme import, new peroxisome membrane formation and division of existing peroxisomes. Defects in 13 genes result in a ZSD phenotype due to the common end-effect of reduced peroxisome enzyme functions and correlates best to the B-oxidation defects and accumulation of toxic products. In contrast, the distinct RCDP phenotype is due to a single pathway deficit in the synthesis of specialized membrane lipids, plasmalogens. In this lecture we will review peroxisome clinical, biochemical and molecular pathways, their relation to ZSD and RCDP phenotypes, new information on these disorders, single enzyme defects of peroxisome function that relate to PBD and rational management approaches. We will emphasize how scientists, physicians and families have worked together in a rare disease, to push the field forward.

### E4.2

#### The value of functional assays in peroxisomal disorders

*H. R. Waterham;*

*Academic Medical Center at the University of Amsterdam, Amsterdam, Netherlands.*

Peroxisomes are dynamic organelles, which, in humans, play an essential role in a variety of cellular catabolic and anabolic metabolic pathways, including fatty acid alpha- and beta-oxidation, and plasmalogen and bile acid synthesis. Defects in genes encoding peroxisomal proteins can result in a large variety of peroxisomal disorders affecting either specific metabolic pathways, i.e. the single peroxisomal enzyme deficiencies, or causing a generalized defect in function, assembly and maintenance of peroxisomes, i.e. peroxisome biogenesis disorders. Clinically, patients with peroxisomal disorders may present with variable severity ranging from early lethality to subtle neurosensory aberrations.

Up till recently, laboratory diagnosis of peroxisomal disorders primarily relied on metabolite analysis followed by biochemical testing and establishment of the underlying gene defect. The increasing implementation of Next Generation Sequencing (NGS) technology as first choice of diagnostic evaluation is changing the diagnostic landscape. While this approach should readily identify genetic defects in classical peroxisomal disorders, it may result in the identification of genetic variants with unknown consequences for peroxisomal functioning and short- or long-term pathogenicity in variant

disorders or in patients with mild disease. Insight into the possible of such DNA variants on metabolism and, as an extension thereof, on the individual requires a multidisciplinary approach combining genetic, biochemical and molecular biological studies. I will briefly review clinical, biochemical and genetic aspects of the different human peroxisomal disorders known to date, with emphasis on some recently discovered defects and including examples of cases where functional assays have been imperative in the final diagnosis.

## E5 Ciliopathies

### E5.1

#### Cilia in human developmental anomalies

*E. Valente;*

*University of Salerno, Salerno, Italy.*

The primary cilium is a solitary organelle protruding from the surface of almost all vertebrate cell types. Long considered a vestigial remnant, this organelle has recently become the focus of intensive research, that demonstrated its many roles in sensing the extracellular environment, and in coordinating key signaling pathways. In particular, during the embryonic development, the primary cilium is known to critically regulate main pathways such as Sonic Hedgehog, Wnt and planar cell polarity, that are implicated in left-right axis formation, limb development and neurogenesis. Thus, it is not surprising that mutations in genes encoding ciliary proteins underlie a wide spectrum of "ciliopathies", that are characterized by substantial overlap among distinct conditions. Indeed, ciliopathies such as Joubert syndrome (JS), Meckel syndrome (MKS), Bardet-Biedl syndrome (BBS), Senior-Loken syndrome (SLS), some oral-facial-digital syndromes (OFD), Jeune asphyxiating thoracic dystrophy (JATD) and other short rib polydactylies, may variably share features such as retinal dystrophy, nephronophthisis or cystic dysplastic kidneys, congenital liver fibrosis, polydactyly and other skeletal abnormalities, situs inversus, occipital encephalocele and midline oral and facial defects. Clinical heterogeneity is mirrored by genetic heterogeneity, with each ciliopathy being caused by mutations in several genes and, on the other hand, mutations in one gene causing a wide phenotypic spectrum, often encompassing different syndromes of variable severity. For instance, JS and the lethal MKS ciliopathy are allelic at 9 loci. Similarly, mutations in three genes (CSPP1, KIAA0586 and CEP120) cause a spectrum of phenotypes with overlapping features of JS, MKS, JATD and OFD. The mechanism through which mutations in the same gene may cause such wide phenotypic variability remains unexplained, with genotype-phenotype correlates only partly explaining this variability. The identification of genetic modifiers and other factors able to influence the penetrance and expression of ciliary mutations, represents one of the biggest challenges of current research on ciliopathies.

## E6 Cost-effectiveness in genetic testing

### E6.1

#### Cost-effectiveness of diagnostics in rare diseases

*K. Payne;*

*Manchester Centre for Health Economics, The University of Manchester, Manchester, United Kingdom.*

The existence of finite healthcare budgets and the scarcity of healthcare resources mean that there is an opportunity cost for every decision made to introduce a new healthcare package, medicine, surgical procedure or diagnostic test into clinical practice. The decision to allocate resources to a particular healthcare intervention excludes those resources from alternative possible uses within a healthcare system. Therefore, ideally, robust evidence is required to inform decision-makers allocating healthcare resources about the potential costs and benefits of incorporating a healthcare intervention within clinical practice. Cost-effectiveness analysis offers a potential framework to evaluate the relative costs and benefits of healthcare interventions compared with current practice. Performing cost-effectiveness analysis of diagnostics for rare diseases may be problematic for many reasons. The aim of this presentation is to describe the key challenges (methodological, practical, technical and organisational) when conducting robust cost effectiveness analysis of diagnostic tests for rare diseases. The presentation will begin by presenting an overview of methods of cost effectiveness analysis and explain how they are used by decision-making bodies such as the National Institute for Health and Care Excellence. Diagnostic tests for rare diseases will then be described using the framework of a complex intervention. The key challenges of performing a cost-effectiveness analysis of

example diagnostic tests will then be described, and illustrated with applied examples, under the following four headings: methodological; practical; technical and organisational. The presentation will conclude by describing the further work required to ensure the methods and practical approaches to perform cost effectiveness analyses of diagnostic tests for rare diseases are sufficiently robust to inform their introduction into clinical practice and maximise the potential benefits for patient populations.

## E7 Cleaning the noise from Big Data

### E7.1

#### Removing unwanted variation in RNA-seq data

*T. Speed<sup>1,2</sup>*

<sup>1</sup>Melbourne, Australia, <sup>2</sup>Walter & Eliza Hall Institute of Medical Research, Parkville, Australia.

Like other kinds of 'omic data, RNA-seq data can be affected by unwanted variation. This is often called batch effects, and includes variation due to time, space, technology, reagents, operators, environmental conditions, and unwanted biological variation, such as between biological replicates. Standard normalization methods for such data may not deal adequately with all unwanted variation. In this talk I will explain and illustrate how methods developed for microarray data can be modified to be effective at removing unwanted variation from RNA-seq data. We make use of negative controls and, when available, replicates. The methods will be illustrated with RNA-seq libraries made from brain tissue. The results discussed are all from joint work with Davide Risso, Lucia Peixoto and others to be named.

## E8 Clinical interpretation of genetic variants

### E8.1

#### Methods for the assessment of variant pathogenicity in cardiac diseases

*S. Cook*

Singapore, China.

The accurate interpretation of genetic variation in Mendelian disease genes has lagged behind data generation as sequencing has become increasingly accessible. Ongoing large sequencing efforts present huge interpretive challenges, but also provide an opportunity to characterize the range and importance of rare variation. In this presentation I will discuss the analysis of sequence data from 7,855 clinical cardiomyopathy cases and 60,706 ExAC reference samples that we used to better understand genetic variation in cardiomyopathy that causes heart failure and sudden death. The analyses show that in some genes previously reported as important causes of a given cardiomyopathy, rare variation is not clinically informative whereas, in other genes, diagnostic laboratories appear overly conservative when assessing variant pathogenicity. The impact of cardiomyopathy research studies in the era of comprehensive sequencing is also discussed. The data point to analytical approaches to evaluate which genes and variant classes are interpretable for cardiomyopathy and may be applied to other multi-allelic Mendelian diseases.

### E8.2

#### Recommendations for the Interpretation of Genetic Variants from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

*H. Rehm*

Laboratory for Molecular Medicine, Partners Personalized Medicine; The Broad Institute of Harvard and MIT; Brigham & Women's Hospital; Harvard Medical School, Boston, MA, United States.

With the plummeting cost of sequencing, genetic data is becoming increasingly available for use in the diagnosis, treatment and prediction of disease. However, robust and accurate use of genomics in the practice of medicine requires high quality knowledgebases and the accurate interpretation of DNA variation. To address these needs, several efforts have been launched. The American College of Medical Genetics and Genomics (ACMG), in collaboration with the Association for Molecular Pathology (AMP), has published a guideline to enable a more systematic assessment of evidence for and against pathogenicity of DNA variants for Mendelian, or monogenic, disease. This includes recommendation for the use of standard terms: 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign' to de-

scribe variants related to monogenic diseases. Variants are classified into these five categories based on combinations of criteria of varying strength (e.g. supporting, moderate, strong, very strong) and type (e.g. population data, computational data, functional data, segregation data, etc.) These guidelines have now been tested by the NIH Clinical Sequencing Exploratory Research Consortium and implemented by many clinical laboratories and commercial platforms. In addition, clinical laboratories and expert working groups operating within the NIH funded Clinical Genome Resource Program (ClinGen) are applying the ACMG/AMP guidelines to resolve differences in variant interpretation identified in the NCBI's ClinVar database and build more granularity into the criteria using gene and disease specific parameters to enable expert review of variants in ClinVar. As of Apr. 2016, over 500 laboratories had submitted over 125,000 unique interpreted variants to ClinVar. Prior analyses in May 2015 showed that ~11% of variants had interpretations submitted by more than one laboratory, and of those ~17% were interpreted differently. Through pilot efforts the majority of these differences in variant interpretation appear to be resolvable and efforts are underway to resolve these differences using the ACMG/AMP guidelines.

## CONCURRENT SESSIONS

## C01 Reproductive Genetics

### C01.1

#### Genome-wide haplotyping of preimplantation embryos in the clinic: principles guiding embryo selection in Leuven

*K. Van Den Bogaert<sup>1</sup>, E. Dimitriadou<sup>1</sup>, C. Melotte<sup>1</sup>, S. Debruck<sup>2</sup>, K. Devriendt<sup>1</sup>, T. de Ravel<sup>1</sup>, T. D'Hooghe<sup>2</sup>, J. R. Vermeesch<sup>1</sup>*

<sup>1</sup>Centre for Human Genetics, UZLeuven, Leuven, Belgium, <sup>2</sup>Leuven University Fertility Centre, UZLeuven, Leuven, Belgium.

Recently, we developed haplarmhisis, a method enabling accurate concurrent haplotyping and copy number profiling and we demonstrated its validity in preimplantation genetic diagnosis for embryo analysis. The introduction in a diagnostic setting raised novel ethical questions. Here we present the principles guiding embryo selection and prioritization that are applied at our center according to the chromosomal content and mutational load of the embryos. Our embryo selection principles are based not only on technical and biological, but also on ethical criteria and have a profound impact on the organization of PGD operations and on the information that is transferred amongst the genetic unit, the fertility clinic and the patients. Those principles are also important for the organization of pre- and post-counselling and influence the way of interpreting and reporting preimplantation genotyping results.

From June 2014 until November 2015, 314 embryos from 50 couples have been tested in 82 cycles, leading to 16 clinical pregnancies (36% clinical pregnancy rate per cycle for which embryo transfer has been finalized) and the birth of 5 healthy babies, so far. Thirty-one different indications, i.e. 27 for monogenic disorders, 3 chromosomal aberrations and 1 case of combined monogenic disorder with chromosomal aberration have been included. As novel genome-wide approaches for embryo selection are revolutionizing the field of reproductive genetics, national and international discussions to set general principles are warranted.

### C01.2

#### Mosaic embryos can achieve good IVF success rates

*R. Morales<sup>1</sup>, B. Lledó<sup>1</sup>, J. Ortiz<sup>1</sup>, H. Blanca<sup>1</sup>, J. Llácer<sup>2</sup>, R. Bernabeu<sup>2</sup>*

<sup>1</sup>Instituto Bernabeu Biotech, Alicante, Spain, <sup>2</sup>Instituto Bernabeu, Alicante, Spain.

**Introduction:** Classification of mosaic embryos as aneuploid embryos is a controversial topic, since the association between mosaicism in trophoectoderm cells and inner mass cells is unknown. Moreover, it seems that there is a mechanism by which mosaicism could be corrected. To clarify this issue the outcomes of transfer cycles with mosaic and euploid blastocysts were compared.

**Material and Methods:** We retrospectively reanalyzed array-CGH results from trophoectoderm biopsies of 816 blastocysts. We considered a mosaic embryo when the percentage of mosaicism, calculated by the log2 ratio, was higher than 25%. Array-CGH analysis was performed using Agilent SurePrint G3 8x60K CGH microarrays with previous whole genome amplification of genomic DNA. The main outcome measures were implantation rate, pregnancy rate and biochemical and clinical miscarriage rate.

**Results:** We detected chromosomal mosaicism in 107 blastocysts (13.1%). Moreover, 49.4% of the analysed embryos were euploid, 30.9% aneuploid

and the remaining 6.6% without diagnosis. In the mosaic group, 57.9% were euploid embryos with mosaicism and 42.1% were also aneuploid. The outcomes of the cycles were compared between cycles where only mosaic embryos were transferred and cycles where euploid embryos were transferred. Significant differences were observed in the clinical pregnancy rate (20.6% in mosaic group vs 38.9% in euploid group;  $p=0.042$ ).

**Conclusions:** Our data show that the transfer of mosaic embryos affects the outcomes of the IVF cycles. Even so, the mosaic embryos have a relatively good IVF success rate and therefore they should not be discarded in couples that don't have euploid embryos for transfer.

## C01.3

### Gonadic mosaicism and prenatal diagnosis options: insights from retinoblastoma

**C. Houdayer<sup>1</sup>, C. Dehainault<sup>2</sup>, L. Golmard<sup>2</sup>, A. Charpin<sup>2</sup>, A. Laugé<sup>2</sup>, I. Aerts<sup>3</sup>, N. Cassoux<sup>4</sup>, G. A. Millot<sup>5</sup>, D. Stoppa Lyonnet<sup>1</sup>, M. Gauthier Villars<sup>6</sup>;**

<sup>1</sup>Institut Curie, oncogenetics, INSERM U830 and Paris Descartes Sorbonne Paris Cité University, Paris, France, <sup>2</sup>Institut Curie, oncogenetics, Paris, France, <sup>3</sup>Département d'oncologie pédiatrique, adolescents jeunes adultes, Institut Curie, Paris, France,

<sup>4</sup>Département d'oncologie chirurgicale, service d'Ophtalmologie, Institut Curie and Université Paris Descartes, Sorbonne Paris Cité, Paris, France, <sup>5</sup>Institut Curie, CNRS UMR 3244 and Sorbonne Universités, UPMC Univ Paris 06, Paris, France.

Mosaicism impacts on the recurrence risk. In sporadic cases, a post-zygotic event signifies a somatic mosaicism in the affected child and thus parents and siblings are freed from follow up. On the other hand, a pre-zygotic mutation transmitted by an unaffected mosaic parent implies recurrence risk in future offspring and thus prenatal diagnosis options might be discussed. To better estimate the contribution of pre- and post-zygotic events, we used sporadic retinoblastoma as a model because 80% of Rb cases are due to de novo mutations.

We analysed 124 consecutive bilateral retinoblastoma probands carrying a heterozygous RB1 mutation previously ascertained by Sanger sequencing and their unaffected, non-carrier parents. In order to evaluate somatic mosaicism in blood, the deleterious mutation identified in the proband was searched for in the trios using targeted deep sequencing. Observed recurrences, which should represent an estimate of germline and somatic mosaicism, were recorded for the 124 sibships.

Deep sequencing evidenced one mosaic unaffected parent out of 124 tested couples, i.e. a 0.8 % risk of mosaicism and thus a theoretical 0.4% risk of recurrence. Follow up in the sibships showed one recurrence out of 199 novel births, i.e. an observed 0.5 % recurrence risk. Similar estimates were obtained by two independent methods, which is convincing. Consequently a mean 0.5% risk could be used or, more cautiously, a maximum recurrence risk of 2 out of 124 i.e. 1.6%. We believe these results could be considered for genetic counseling in other diseases with a high de novo mutation rate.

## C01.4

### Zygotes segregate entire parental genomes in distinct blastomere lineages causing cleavage stage chimaerism and mixoploidy

**A. Destouni<sup>1</sup>, M. Zamani Esteki<sup>2</sup>, M. Catteeuw<sup>3</sup>, O. Tšuiko<sup>1,4</sup>, E. Dimitriadou<sup>1</sup>, K. Smits<sup>3</sup>, A. Kurg<sup>4</sup>, A. Salumets<sup>5,6</sup>, A. Van Soom<sup>7</sup>, T. Voet<sup>\*\*8,2,7</sup>, J. R. Vermeesch<sup>\*\*1</sup>, \*Joint first-authors, \*\*Co-senior authors;**

<sup>1</sup>Laboratory of Cytogenetics and Genome Research, Center of Human Genetics, KU Leuven, Leuven, Belgium, <sup>2</sup>Laboratory of Reproductive Genomics, Center of Human Genetics, KU Leuven, Leuven, Belgium, <sup>3</sup>Department of Obstetrics, Reproduction and Herd Health, Ghent University, Ghent, Belgium, <sup>4</sup>Institute of Molecular and Cell Biology, Tartu University, Tartu, Estonia, <sup>5</sup>Competence Centre on Health Technologies, Tartu, Estonia, <sup>6</sup>Department of Obstetrics and Gynecology, University of Tartu, Tartu, Estonia, <sup>7</sup>Sanger-EBI Single Cell Genomics Centre, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

**Introduction:** Chimaerism and mixoploidy are defined by the presence of cell lineages with different parental genomes or different ploidy states in a single individual, respectively. However, our knowledge on their mechanistic origin is limited, as it results from indirect observations, often when the cell lineages have been subject to rigorous selective pressure during development.

**Materials and Methods:** We applied haplithesis on SNP genotype data to infer the haplotypes and the copy number of parental genomes in 116 single blastomeres comprising entire in vitro produced preimplantation bovine embryos ( $n=23$ ). Haplithesis uncovers copy number aberrations and determines their mechanistic/segregational origin at the single-cell level in unprecedented detail, enabling reliable reconstruction of the each embryo's cleavage history back to the zygote stage.

**Results:** We demonstrate that chromosome instability is comparable between bovine and human cleavage embryos. We further uncover a novel form of genomic instability whereby zygotes spontaneously segregate entire pa-

rental genomes into different cell lineages during the first post-zygotic cleavage division. Importantly, we observed that parental genome segregation was not exclusively triggered by abnormal dispermic fertilization, but also normally fertilized zygotes can spontaneously segregate entire parental genomes into distinct cell lineages during cleavage of the zygote. Preliminary data show the mechanism to be conserved in humans as well.

**Conclusions:** We discover a novel mechanism of aberrant cell division in the zygote culminating with the segregation of the parental genomes into distinct cell lineages. We coin this "Heterogoneic" cell division. This mechanism is likely the main cause for chimaerism and mixoploidy in mammals.

## C01.5

### Differential expression of parental alleles of BRCA1 in human preimplantation embryos

**P. Tulay, 99138<sup>1,2</sup>, A. Doshi<sup>3</sup>, P. Serhal<sup>3</sup>, S. B. SenGupta<sup>2</sup>;**

<sup>1</sup>Near East University, Nicosia, Cyprus, <sup>2</sup>UCL, Institute for Women's Health, London, United Kingdom, <sup>3</sup>The Centre for Reproductive and Genetic Health, London, United Kingdom.

**Introduction:** The expression of parental genomes is required for completion of embryogenesis. Differential methylation of each parental genome has been observed in mouse and human preimplantation embryos. It is possible that differences in methylation affect the level of gene transcripts from each parental genome in early developing embryos. The aim of this study was to investigate if there is a parent specific pattern of *BRCA1* expression in human embryos and to examine if this affects embryo development when the embryo carries a *BRCA* mutation.

**Materials and Methods:** Differential parental expression of *ACTB*, *SNRPN*, *H19* and *BRCA1* was semi-quantitatively analysed by mini-sequencing in 95 human preimplantation embryos obtained from couples undergoing preimplantation genetic diagnosis (PGD).

**Results:** *BRCA1* was shown to be differentially expressed favouring the paternal transcript in early developing embryos. Methylation specific PCR showed a variable methylation profile of *BRCA1* promoter region at different stages of embryonic development. Embryos carrying paternally inherited *BRCA* mutations were shown to develop more slowly compared to the embryos with maternally inherited *BRCA* mutations.

**Conclusions:** The results of this study suggest that differential gene expression can influence the early development of preimplantation embryos. When the paternal *BRCA1* transcript present in the embryo carries a mutation, the embryo may become more vulnerable to stress due to rapid demethylation of the paternal genome and the gradual demethylation of the maternal genome. Further extrapolation of this data suggests that the risk of transmitting a *BRCA* mutation may be modulated by the parental origin of the mutation.

## C01.6

### Novel autosomal genes linked with male infertility

**D. V. S. Sudhakar<sup>1</sup>, R. Phanindranath<sup>1</sup>, R. Singh<sup>2</sup>, N. J. Gupta<sup>3</sup>, M. Deenadayal<sup>4</sup>, Y. Sharma<sup>1</sup>, K. Thangaraj<sup>1</sup>;**

<sup>1</sup>Centre for Cellular and Molecular Biology, Hyderabad, India, <sup>2</sup>Central Drug Research Institute, Lucknow, India, <sup>3</sup>Institute of Reproductive Medicine, Kolkata, India, <sup>4</sup>Infertility institute and research center, Hyderabad, India.

**Introduction:** Approximately 15% of the couples are infertile worldwide. Impaired fertility of male partner is causative in approximately 50% of infertile couples. Earlier studies from our lab have shown that about 8.5% infertility among Indian men is due to the Y chromosome microdeletions. Further, autosomal and mitochondrial mutations accounted for 20.5% of the genetic factors responsible for infertility among Indian men. However, etiology of large proportion (71%) of infertile men still remained unknown. Therefore, we performed exome sequencing to identify novel genes responsible for male infertility.

**Materials and methods:** we sequenced exome of 44 idiopathic infertile men using Illumina Hiseq-2000 platform with 100X coverage. Using various bioinformatics tools, we have identified 32 novel and rare variants and they were further genotyped in 1000 cases and controls. Of the novel genes identified, *CETN1* was found to be a strong candidate; hence we have sequenced complete *CETN1* in 875 infertile and 552 ethnically matched fertile men. Identified variants were characterized using biophysical and cell biology approaches.

**Results:** We have identified a total of 32 novel, stop gain and missense variants from 30 genes. Sequencing of *CETN1* revealed that one 5'UTR (rs367716858) and a missense (rs61734344) variant are associated with male infertility. Functional studies have shown that rs61734344 (p. Met72Thr) alters calcium binding affinity, thermodynamic properties and sur-

face hydrophobicity. Functional analysis of 5'UTR variant (rs367716858) revealed that mutation causes increased expression of Centrin-1 protein. Conclusion: Our study has identified novel autosomal genes linked with male infertility.

## C02 Intellectual Disability

### C02.1

#### FRRS1L Mutations link intellectual disability to altered priming of AMPA-receptor biogenesis

A. Brechet<sup>1</sup>, R. Buchert<sup>2</sup>, J. Schwenk<sup>3,4</sup>, S. Boudkkazi<sup>1</sup>, G. Zolles<sup>1</sup>, K. Squier-Pernet<sup>5</sup>, D. Loretz<sup>1</sup>, I. Schaber<sup>1</sup>, A. Saadi<sup>6</sup>, C. Bole-Feysoz<sup>5</sup>, P. Nitschke<sup>7</sup>, A. Reis<sup>7</sup>, H. Sticht<sup>8</sup>, N. Al-Sanna<sup>9,10</sup>, A. Rolfs<sup>10</sup>, A. Kulik<sup>2</sup>, U. Schulte<sup>1,4</sup>, R. Abou-Jamra<sup>7,11</sup>, L. Colleaux<sup>12</sup>, B. Fakler<sup>1,4</sup>; <sup>1</sup>Institute of Physiology, University of Freiburg, Freiburg, Germany, <sup>2</sup>Institute of Human Genetics, University of Erlangen, Erlangen, Germany, <sup>3</sup>Institute of Physiology, University of Freiburg, Freiburg, Germany, <sup>4</sup>Center for Biological Signaling Studies (BIOS), Freiburg, Germany, <sup>5</sup>Institut IMAGINE INSERM U1163, Paris, France, <sup>6</sup>Department de Neurologie, Etablissement Hospitalier Spécialisé de Benaknoun, Algiers, Algeria, <sup>7</sup>Institute of Human Genetics, University of Erlangen, Erlangen, Germany, <sup>8</sup>Institute of Biochemistry, Emil-Fischer Center, Erlangen, Germany, <sup>9</sup>Dharaan Health Center, Saudi Aramco, Saudi Arabia, <sup>10</sup>Albrecht-Kosell-Institute for Neuroregeneration, Medical University Rostock, Rostock, Germany, <sup>11</sup>Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Germany, <sup>12</sup>Institut IMAGINE INSERM U1163, Paris, France.

Identifying causes of intellectual disability remains a considerable medical challenge. Here, we report on six patients from three unrelated consanguineous Algerian, Syrian and Saudi Arabian families presenting with a core phenotype of moderate to severe intellectual disability, speech delay and seizures. High-throughput sequencing identified three distinct homozygous mutations in the FRRS1L gene: a missense variant (p.Lys155Glu), a one base-pair deletion (p.Val195Glufs\*35) and a nonsense mutation (p.Gln321\*). FRRS1L encodes a previously uncharacterized peripheral constituent of the AMPA glutamate receptors (AMPARs). AMPARs are key elements of the mammalian brain responsible for a variety of processes including fast excitatory neurotransmission, postsynaptic plasticity, or synapse development. These macromolecular complexes assemble from more than 30 different constituents. The inner core determines the biophysical properties of the receptors while the periphery is involved in various aspects of synapse physiology.

To further characterize the physiological role of FRRS1L upon brain development and function, we used reverse proteomics, biochemical, morphological and functional analyses to identify and characterize FRRS1L-containing AMPARs assemblies.

We demonstrated that AMPAR complexes containing FRRS1L are restricted to the endoplasmic reticulum and lack the inner core constituents of AMPARs at the plasma membrane. Virus-directed deletion and overexpression of FRRS1L in adult rats alters the number of AMPARs in individual synapses resulting in markedly reduced or increased amplitudes of the EPSCs without effects on their time courses

Our data identify FRRS1L as a new neurodevelopmental-disease gene and highlight the key role of this gene in the priming step of AMPAR biogenesis and fast excitatory synaptic transmission.

### C02.2

#### PIGG: a novel gene causing intellectual disability, seizures and hypotonia

P. Makrythanasis<sup>1,2</sup>, M. Kato<sup>3</sup>, M. Zaki<sup>4</sup>, H. Saito<sup>5</sup>, K. Nakamura<sup>6</sup>, F. A. Santoni<sup>1,2</sup>, S. Miyatake<sup>7</sup>, M. Nakashima<sup>8</sup>, M. Y. Issa<sup>9</sup>, M. Guipponi<sup>2</sup>, A. Letourneau<sup>1</sup>, C. Logan<sup>7</sup>, N. Roberts<sup>7</sup>, D. A. Parry<sup>7</sup>, C. A. Johnson<sup>7</sup>, N. Matsumoto<sup>5</sup>, H. Hamamy<sup>1</sup>, E. Sheridan<sup>8</sup>, T. Kinoshita<sup>3</sup>, S. E. Antonarakis<sup>1,2,9</sup>, Y. Murakami<sup>2</sup>;

<sup>1</sup>Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland, <sup>2</sup>Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland, <sup>3</sup>Department of Immunoregulation, Research Institute for Microbial Diseases, and World Premier International Immunology Frontier Research Center, Osaka University, Osaka, Japan, <sup>4</sup>Department of Clinical Genetics, National Research Centre, Cairo, Egypt, <sup>5</sup>Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan, <sup>6</sup>Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan, <sup>7</sup>School of Medicine, University of Leeds, Leeds, United Kingdom, <sup>8</sup>School of Medicine, University of Leeds, L, United Kingdom, <sup>9</sup>iGE3, Institute of Genetics and Genomics of Geneva, University of Geneva, Geneva, Switzerland.

Glycosylphosphatidylinositol (GPI) is a glycolipid that anchors >150 various proteins to the cell surface. At least 27 genes are involved in biosynthesis and transport of GPI anchored proteins (GPI-APs). To date mutations in 13 of these genes are known to cause inherited GPI deficiencies (IGDs); all inherited as recessive traits. IGDs mainly show intellectual disability, epilepsy, coarse facial features and multiple organ anomalies. Those sym-

ptoms are caused by the decreased surface expression of GPI-APs or by structural abnormalities of GPI. Here we present five affected individuals from three families, two consanguineous from Egypt and Pakistan and one from Japan showing intellectual disability, hypotonia and early onset seizures. We identified pathogenic variants in PIGG, a GPI pathway gene. In the consanguineous families, homozygous variants, c.928C>T:p.(Gln310\*) and c.2261+1G>C were found, while the Japanese individual was compound heterozygous for c.2005C>T: p.(Arg669Cys) and a 2.4Mb deletion that involved PIGG. PIGG is the enzyme that modifies the second mannose with ethanolamine phosphate, which is removed soon after GPI is attached to the protein. Physiological significance of this transient modification has been unclear. Using B lymphoblasts from affected individuals of families from Egypt and Japan, we revealed that PIGG activity was almost completely abolished; however the GPI-APs were normally expressed on the surface with the normal structure, indicating that the pathogenesis of PIGG deficiency is not yet fully understood. The discovery of pathogenic variants in PIGG expands the spectrum of IGD, and further enhances our understanding of this etiopathogenic class of intellectual disability.

### C02.3

#### De novo mutations in Histone 3 Family 3B are associated with a severe neurodegenerative disorder and brain atrophy

A. Telegraf<sup>1</sup>, E. F. Joiner<sup>2</sup>, M. T. Cho<sup>1</sup>, K. Retterer<sup>1</sup>, J. L. Schutte<sup>3</sup>, J. W. Innis<sup>3</sup>, B. Fregeau<sup>4</sup>, E. H. Sherr<sup>4</sup>, A. Pizzino<sup>5</sup>, A. Vanderver<sup>5</sup>, S. Lüttgen<sup>6</sup>, J. Denecke<sup>7</sup>, T. M. Strom<sup>8,9</sup>, D. Lessel<sup>6</sup>, J. Juiusola<sup>1</sup>, K. G. Monaghan<sup>1</sup>, W. K. Chung<sup>10,11</sup>, <sup>1</sup>GeneDx, Gaithersburg, MD, United States, <sup>2</sup>Department of Pediatrics, Columbia University Medical Center, New York, NY, United States, <sup>3</sup>Departments of Human Genetics and of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI, United States, <sup>4</sup>Department of Neurology, University of California, San Francisco, San Francisco, CA, United States, <sup>5</sup>Children's National Health System, Washington, DC, United States, <sup>6</sup>Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, <sup>7</sup>Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, <sup>8</sup>Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany, <sup>9</sup>Institute of Human Genetics, Technische Universität München, Munich, Germany, <sup>10</sup>Department of Pediatrics, Columbia University Medical Center, New York, NY, United States, <sup>11</sup>Department of Medicine, Columbia University Medical Center, New York, NY, United States.

We describe four patients with a severe and progressive neurological phenotype characterized by developmental delays, seizures, and brain atrophy due to de novo predicted pathogenic variants in the Histone 3 Family 3B (H3F3B) gene (MIM # 601058). H3F3B gene encodes histone variant H3.3, a displacement histone critical to neuronal and glial development and transcription as well as formation and maintenance of synaptic connectivity in the brain. Clinical whole exome sequencing (WES) was performed on 3,235 patients with developmental delay or intellectual disability. Four affected individuals from four unrelated proband-parent trios were heterozygous for a de novo missense H3F3B variant. All variants were absent from NHLBI Exome Sequencing Project, 1000 Genomes, Exome Aggregation Consortium public databases and our internal WES database of unaffected individuals. All variants occurred within highly conserved domains, and were predicted to be damaging or possibly damaging from multiple *in silico* models. The patients range in ages from 22 months to 13 years old. The clinical phenotype is dominated by developmental delay, intellectual disability, seizures, with microcephaly and brain atrophy present in the three oldest children. Brain abnormalities are characterized by cortical atrophy and white matter abnormalities in all three; whereas thin corpus callosum in one child. Additional features include tone abnormalities, aspiration, gastroesophageal reflux, vision or oculomotor disturbances. To our knowledge, the findings presented in this case series are the first germline variants in this gene associated with a neurological phenotype and may facilitate the identification of additional affected patients to further characterize this novel neurodegenerative disorder.

### C02.4

#### Autosomal recessive mutations of the neuron specific $\beta$ 3B subunit of clathrin-associated adaptor protein complex 3 (AP3B2) cause an early onset epileptic encephalopathy with optic atrophy

M. Assoum<sup>1</sup>, C. Philippe<sup>2</sup>, B. Isidor<sup>3</sup>, L. Perrin<sup>4</sup>, P. Makrythanasis<sup>5,6</sup>, N. Sondheimer<sup>7</sup>, C. Paris<sup>8</sup>, S. Antonarakis<sup>9,10</sup>, H. Hamamy<sup>11</sup>, T. Jouan<sup>1</sup>, Y. Duffourd<sup>1,12</sup>, M. Milh<sup>13,14</sup>, S. Auvin<sup>15</sup>, K. Mireskandari<sup>16</sup>, J. Rivière<sup>1,12,17</sup>, L. Faivre<sup>18,12</sup>, J. Thevenon<sup>1,10,12</sup>, <sup>1</sup>Equipe d'Accueil 4271, Génétique des Anomalies du Développement, Université de Bourgogne, dijon, France, <sup>2</sup>Laboratoire de Génétique Médicale, INSERM U954, CHU de Nancy, Nancy, France, <sup>3</sup>Service de Génétique Médicale, Inserm UMR-S 957, CHU Nantes, Nantes, France, <sup>4</sup>Département de Génétique, Assistance Publique – Hôpitaux de Paris (AP-HP) – CHU Paris - Hôpital Robert Debré, Paris, France, <sup>5</sup>Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland, <sup>6</sup>Service of

*Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland, <sup>7</sup>The Hospital for Sick Children, Toronto, Canada, Toronto, ON, Canada, <sup>8</sup>Centre Hospitalier Régional Universitaire, Hôpital Jean Minjoz, besançon, France, <sup>9</sup>Division of Genetic Medicine, Geneva University Hospitals, Geneva, Switzerland, <sup>10</sup>iGE3, Institute of Genetics and Genomics of Geneva, University of Geneva, Geneva, Switzerland, <sup>11</sup>Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland, <sup>12</sup>Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (TRANSLAD); Centre Hospitalier Universitaire Dijon, Dijon, France, <sup>13</sup>Service de Neurologie Pédiatrique, Hôpital de la Timone, Assistance Publique des Hôpitaux de Marseille, Marseille, France, <sup>14</sup>Unité Mixte de Recherche 910, Institut National de la Santé et de la Recherche Médicale, Aix-Marseille Université, Marseille, France, <sup>15</sup>Service de neurologie pédiatrique Inserm 1141, Hôpital Robert Debré, Paris, France, <sup>16</sup>The Hospital for Sick Children, Department of Ophthalmology and Vision Sciences, Toronto, ON, Canada, <sup>17</sup>Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs de l'Interrégion Est, Centre Hospitalier Universitaire Dijon, Dijon, France, <sup>18</sup>Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs de l'Interrégion Est, CHU Dijon, Dijon, France.*

Early-onset epileptic encephalopathies (EOEE) represent a heterogeneous group of severe disorders characterized by severe seizures, interictal epileptiform activity on a disorganized EEG background, developmental regression or retardation, and onset before one year of age. Among a cohort of 50 patients with EE, we ascertained two unrelated patients with EOEE associated with severe developmental impairment and autosomal recessive variants (namely missense changes and splice-site variants) of AP3B2 by means of whole-exome sequencing. The targeted sequencing of AP3B2 in 86 additional unrelated individuals with EOEE led to the identification of an additional family with two affected children carrying a homozygous 4-bp deletion leading to a frameshift and premature stop-codon. Two additional families with four affected individuals were gathered through the Matchmaker Exchange initiative by matching on autosomal recessive mutations of AP3B2. This report presents eight individuals from five unrelated families presenting with global hypotonia, an EOEE with stagnation or regression of psychomotor acquisitions with absent speech. Eye contact was poor and associated with an optic atrophy when assessed (5/5). Patients presented with a post-natal microcephaly (7/8) and brain MRI identified a superior cerebellar vermis atrophy. AP3B2 encodes for a neuron specific subunit of the AP-3B complex, which is involved in synaptic vesicles biogenesis and transport. Conversely, a natural *ap3b2* knockout mouse strain was reported with neurodevelopmental disorders including tonic-clonic seizures. To conclude, these genetic evidences support the implication of autosomal recessive mutations of AP3B2 in EOEE with optic atrophy.

## C02.5

### *De novo germline mutations of mTOR pathway genes RHEB and RAC1 cause developmental phenotypes with alterations in brain size*

*M. R. F. Reijnders<sup>1</sup>, M. Kousi<sup>2</sup>, G. M. van Woerden<sup>3</sup>, G. M. Mancini<sup>4</sup>, T. van Essen<sup>5</sup>, C. Marcelis<sup>1</sup>, M. Proietti-Onori<sup>3</sup>, M. van Gastel<sup>6</sup>, E. Smeets<sup>7</sup>, S. J. C. Stevens<sup>7</sup>, C. Gilissen<sup>1</sup>, R. Pfundt<sup>8</sup>, P. L. Tan<sup>2</sup>, T. Kleefstra<sup>1</sup>, Y. Elgersma<sup>9</sup>, N. Katsanis<sup>10</sup>, H. G. Brunner<sup>11</sup>, <sup>1</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>Center for Human Disease Modeling, Duke University, Durham, NC, United States, <sup>3</sup>Department of Neuroscience and ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus University Medical Center, Rotterdam, Netherlands, <sup>4</sup>Department of Clinical Genetics, Erasmus MC, Sophia Children's Hospital, Rotterdam, Netherlands, <sup>5</sup>University of Groningen, University Medical Center of Groningen, Department of Genetics, Groningen, Netherlands, <sup>6</sup>Department of medical care, SWZ zorg, Son, Netherlands, <sup>7</sup>Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, Netherlands.*

One of the pathways known to regulate cell growth is the mTOR pathway through the MTORC1 and MTORC2 complex. Somatic mutations in genes within the mTOR pathway are a well-known cause of hemimegalencephaly in humans by upregulating mTOR activity. Subsequently, germline mutations in mTOR pathway genes might also affect brain size. Here, we report germline *de novo* missense mutations in the mTOR activators RHEB (2 families, 3 patients) and RAC1 (2 families, 2 patients). Mutations in both mTOR pathway genes cause an intellectual disability (ID) syndrome with aberrant head and brain size. Whereas patients with RHEB mutations show severe ID with hypotonia, epilepsy and macrocephaly, microcephaly was observed in patients with RAC1 mutations. Concordant with the human phenotype, we found that overexpression of mutant *rheb* in zebrafish embryos results in megalencephaly and increased neural proliferation, while overexpression of mutant *rac1* causes microcephaly, decreased neuronal proliferation and cerebellar defects. Intrauterine transfer of mutant *Rheb* into the brain of embryonic mice disrupted neuronal migration and caused epilepsy. Neurocognitive phenotypes observed in zebrafish carrying mutant RHEB peptides could be ameliorated through pharmacological treatment with the mTOR pathway antagonist rapamycin, providing clues for potential future therapeutic targets in patients with activating *de novo* RHEB mutations. Based on our genetic and functional data *de novo* germline mutations of mTOR

pathway regulator genes RHEB and RAC1 cause ID with opposite alterations in head and brain size, which reflect the functional effect of these mutations during vertebrate development.

## C02.6

### *De novo germline mutations in exon 5 of PPM1D cause intellectual disability*

*S. Jansen<sup>1</sup>, R. Pfundt<sup>1</sup>, R. Brough<sup>2</sup>, J. C. Herkert<sup>3</sup>, E. J. Marco<sup>4</sup>, M. H. Willemsen<sup>1</sup>, T. Kleefstra<sup>1</sup>, J. A. Veltman<sup>1,5</sup>, C. J. Lord<sup>2</sup>, L. E. L. M. Vissers<sup>1</sup>, B. B. A. De Vries<sup>1</sup>,*

*<sup>1</sup>Donders Centre for Neuroscience, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>The CRUK Gene Function Laboratory and Breast Cancer Now Research Centre, The Institute of Cancer Research, London, United Kingdom, <sup>3</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>4</sup>University of California, San Francisco, CA, United States, <sup>5</sup>Maastricht University Medical Centre, Maastricht, Netherlands.*

*PPM1D* (Protein phosphatase, Mg<sup>2+</sup>/Mn<sup>2+</sup> dependent 1D), or *Wip1*, is a type 2C phosphatase that regulates stress response pathways including the DNA Damage Response, through inhibiting p53 and other tumour suppressors.

Mosaic mutations in non-tumour cell lineages in the last exon (exon 6) of *PPM1D* have been found in patients with breast and ovarian cancer. These truncating mutations, which might have been chemotherapy-induced, retained the phosphatase catalytic domain, leading to a gain-of-function effect. We diagnosed 4 children (2-14 years) with mild-moderate intellectual disability (ID) who had *de novo* germline frameshift, nonsense or splice site mutations in exon 5 of *PPM1D*. Various behavioral and neurological problems were noted, such as ADHD, ODD, anxiety disorder, neonatal feeding problems, hypotonia, gait problems and a high pain threshold. Shared facial dysmorphisms show a broad forehead, lateral hypoplasia of eyebrows, long palpebral fissures, upturned nose, short philtrum and broad mouth.

In fibroblasts and lymphoblastoid cell lines derived from three patients, we tested p53 activation in response to ionizing radiation (IR) exposure. All three cell lines showed normal to increased p53 activation compared to controls, in contrast to the suppression of IR-induced p53 activation previously seen in HeLa cells and U2OS cells transfected with cancer associated truncated *PPM1D* cDNA alleles with exon 6 mutations.

In conclusion, we show that *de novo* germline mutations in exon 5 of *PPM1D* cause syndromic ID. Notably, these mutations do not appear to cause gain-of-function effects on p53 as has been shown for exon 6 mutations in breast and ovarian cancer patients.

## C03 Mutational Mechanisms

### C03.1

#### *Copy number variation morbidity map of congenital limb malformations reveals that the majority of pathogenic variants affect non-coding regulatory elements*

*M. Spielmann<sup>1,2</sup>, R. Flöttmann<sup>1</sup>, B. Kragstein<sup>2</sup>, M. Socha<sup>2</sup>, S. Geuer<sup>2</sup>, L. Allou<sup>2</sup>, A. Sowińska-Seidler<sup>2</sup>, J. Wagner<sup>1</sup>, A. Jamsheer<sup>3</sup>, B. Oehl-Jaschowitz<sup>4</sup>, D. de Silva<sup>5</sup>, I. Kurth<sup>6</sup>, I. Maya<sup>7</sup>, F. Santos<sup>8</sup>, W. Hülsemann<sup>9</sup>, D. Horn<sup>1</sup>, S. Mundlos<sup>1,2</sup>,*

*<sup>1</sup>Charité – Universitätsmedizin Berlin, Berlin, Germany, <sup>2</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>3</sup>Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland, <sup>4</sup>Gemeinschaftspraxis für Humangenetik Homburg/Saar, Berlin/Homburg, Germany, <sup>5</sup>Department of Physiology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka, <sup>6</sup>Institute of Human Genetics, Jena University Hospital, Friedrich-Schiller-University Jena, Jena, Germany, <sup>7</sup>Raphael Recanati Genetics Institute, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel, <sup>8</sup>Pediatrics, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain, <sup>9</sup>Handchirurgie Kinderkrankenhaus Wilhelmstift, Hamburg, Germany.*

Congenital limb malformations can occur as part of a syndrome or as an isolated form. Microarray studies in single affected families have previously demonstrated the importance of copy number variations (CNVs) in limb malformations, but no large scale study has been performed so far and the majority of cases remain undiagnosed. Here we applied high resolution array CGH to 300 patients with congenital limb malformations. We found 31 pathogenic CNVs in known disease loci and identified 6 new loci previously not known to be associated with limb malformations. The pathogenic CNVs affected non-coding cis regulatory elements more frequently than expected (21 non-coding vs 16 coding). We performed functional studies in transgenic mice using the CRISPR/Cas9 system and/or segregation studies in these families to investigate the pathogenicity of 6 novel CNVs causing limb defects. Overall we reached a diagnostic yield of 12%, which is comparable to copy number studies in other cohorts such as intellectual disability. However, the majority of the pathogenic CNVs (57%) were likely to result from changes in the non-coding cis regulatory landscape, while only 43% were due to gene dosage effects or haploinsufficiency. Additionally, we identified 20 rare CNVs of unknown clinical significance (7%) that were inherited

from an unaffected parent. Due to reduced penetrance, a key feature of limb malformations, these rare CNVs might still have an important impact on the skeletal phenotypes of our patients. Our results suggest that CNVs affecting non-coding regulatory elements are a major cause of congenital limb malformations.

### C03.2

#### Microhomology underlies the formation of balanced germline human translocations

*D. Nilsson<sup>1,2,3</sup>, M. Pettersson<sup>1</sup>, P. Gustavsson<sup>1,2</sup>, A. Förster<sup>1</sup>, W. Hofmeister<sup>1</sup>, J. Wincent<sup>1</sup>, V. Zachariadis<sup>1</sup>, B. Anderlid<sup>1,2</sup>, A. Nordgren<sup>1,2</sup>, O. Mäkitie<sup>4,5</sup>, V. Wirta<sup>6,7</sup>, F. Vezzi<sup>7,8</sup>, J. R. Lupski<sup>9,9</sup>, M. Nordenskjöld<sup>1,2</sup>, E. Syk Lundberg<sup>1,2</sup>, C. M. B. Carvalho<sup>9</sup>, A. Lindstrand<sup>1,2</sup>;*  
<sup>1</sup>Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>Karolinska University Hospital, Stockholm, Sweden, <sup>3</sup>SciLifeLab, Stockholm, Sweden, <sup>4</sup>University of Helsinki, Helsinki, Finland, <sup>5</sup>Folkhälsan Institute of Genetics, Helsinki, Finland, <sup>6</sup>KTH Royal Institute of Technology, Stockholm, Sweden, <sup>7</sup>Stockholm University, Stockholm, Sweden, <sup>8</sup>Baylor College of Medicine, Houston, TX, United States, <sup>9</sup>Texas Children's Hospital, Houston, TX, United States.

**Introduction:** Most apparently balanced translocations are thought to result mechanistically from non-homologous end-joining (NHEJ) or, in rare cases of recurrent events, by nonallelic homologous recombination (NAHR). However, few events have been studied with breakpoint resolution.

**Materials and Methods:** Here, we use low coverage mate pair whole genome sequencing to identify and fine map rearrangement breakpoint junctions in individuals with previously detected germ line translocations. In total, 46 junctions from 22 carriers of balanced translocations including both phenotypically normal individuals (n=8) and affected individuals (n=14) were characterized.

**Results:** Genes were disrupted in 48% of the breakpoints; recessive genes in 50% of the normal carriers (inferring a risk of mendelian disease in the offspring) and known dominant intellectual disability genes in 21% of the affected carriers. Finally, seven disrupted candidate disease genes involved in various cellular functions and pathways were identified. Microhomology (2 to 6 bp) was observed in 69% of translocation events. Surprisingly, small insertions (5 to 12 bp), originating from local genomic sequences, were observed in 23%.

**Conclusions:** Microhomology associated with templated-insertions is a characteristic of breakpoint junctions for rearrangements mediated by the error prone replication-based repair mechanisms (RBMs). Our data therefore indicate, that even though NHEJ likely underlie many of the balanced translocations reported here, RBMs contribute to the formation of at least 20% of the cases. The involvement of RBM in these rearrangements supports the contention that a significant portion of so-called germ line translocations may have a mitotic origin.

### C03.3

#### A distinct class of chromoanagenesis events characterized by focal copy number gains

*H. Masset<sup>1</sup>, M. S. Hestand<sup>1</sup>, H. Van Esch<sup>1</sup>, P. Kleinfinger<sup>2</sup>, J. Plaisancier<sup>3</sup>, A. Afenjar<sup>4</sup>, R. Molignier<sup>5</sup>, C. Schluth-Böld<sup>6</sup>, D. Sanlaville<sup>6</sup>, J. R. Vermeesch<sup>1</sup>;*  
<sup>1</sup>KU Leuven, Leuven, Belgium, <sup>2</sup>Laboratoire Cerba, Saint-Ouen l'Aumône, France, <sup>3</sup>Hôpital Purpan, Toulouse, France, <sup>4</sup>Hôpital Armand-Trousseau, Paris, France, <sup>5</sup>Clinique Saint Jean Languedoc, Toulouse, France, <sup>6</sup>Hospices Civils de Lyon, Lyon, France.

**Introduction:** Chromoanagenesis is the process by which a single catastrophic event creates complex rearrangements confined to a single or a few chromosomes. It is usually characterized by the presence of multiple deletions and/or duplications, as well as by copy neutral rearrangements. In contrast, an array CGH screen of patients with developmental anomalies revealed three patients in which a single chromosome carries from 8 to 11 large copy number gains confined to a single chromosome, but the absence of deletions. Surprised by this finding, we set out to further characterize these derivative chromosomes.

**Materials and Methods:** Chromosome structure was evaluated using fluorescence in situ hybridization (FISH), Illumina massive parallel sequencing, and PacBio single-molecule sequencing. Breakpoints were validated by PCR and Sanger sequencing.

**Results:** FISH and Illumina/PacBio sequencing revealed derivative chromosomes maintain their original order, with inserted duplons clustered together in distinct locations. Breakpoint junction sequences showed both microhomology and non-templated insertions of up to 40 bp.

**Conclusions:** Here we present three patients with a single altered chromosome composed of clustered insertional duplications, no deletions, and breakpoint junction sequences showing microhomology and/or non-templated insertions. These observations are difficult to reconcile with current mechanistic descriptions of chromothripsis and chromoanansynthesis. Therefore, we hypothesize those rearrangements to be of a mechanistically different origin. In addition, we suggest that large untemplated insertional sequences

observed at breakpoints are driven by a non-canonical non-homologous end joining mechanism.

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### C03.4

#### Tissue-specific mutation accumulation in human adult stem cells during life

*F. Blokzijl<sup>1</sup>, J. de Ligt<sup>1</sup>, M. Jager<sup>1</sup>, V. Sasseini<sup>1</sup>, S. Roerink<sup>2</sup>, N. Sasak<sup>1</sup>, M. Huch<sup>1</sup>, S. Boymans<sup>1</sup>, P. Prins<sup>1</sup>, I. J. Nijman<sup>1</sup>, I. Martincorena<sup>2</sup>, M. Mokry<sup>3</sup>, C. L. Wiergerinck<sup>3</sup>, S. Middendorp<sup>3</sup>, E. E. S. Nieuwenhuis<sup>3</sup>, M. M. A. Verstegen<sup>4</sup>, L. J. W. van der Laan<sup>4</sup>, J. de Jonge<sup>4</sup>, J. N. M. IJzermans<sup>4</sup>, R. G. Vries<sup>1</sup>, M. van de Wetering<sup>1</sup>, M. R. Stratton<sup>2</sup>, H. Clevers<sup>1</sup>, E. Cuppen<sup>1</sup>, R. van Boxtel<sup>1</sup>;*

<sup>1</sup>Hubrecht Institute for Developmental Biology and Stem Cell Research, KNAW and University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>Cancer Genome Project, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom, <sup>3</sup>Department of Pediatrics, University Medical Center Utrecht, Utrecht, Netherlands, <sup>4</sup>Department of Surgery, Erasmus MC-University Medical Center, Rotterdam, Netherlands.

Gradual accumulation of mutations in human adult stem cells during life is associated with various age-related diseases, including cancer. The number of stem cell divisions throughout life is believed to be a major determinant for mutation accumulation and could explain the extreme variation of cancer incidence across different organs. Yet, mutation patterns and rates of healthy adult stem cells remain unknown.

Here, we determined genome-wide mutation patterns in primary adult stem cells of the small intestine, colon and liver of human donors with ages ranging from 3 to 87 years.

We find that the number of mutations increases linearly with age up to several thousand mutations per cell at 87 years of age, while mutation spectra remain constant throughout life. Small intestine and colon stem cells have a 2-fold higher mutation rate per year compared with liver stem cells. These differences could be exclusively attributed to the mutagenic action of spontaneous deamination of cytosine residues and may reflect the high stem cell division rate in these tissues.

The genomic distribution of somatic mutations is non-random and predominantly associated with DNA replication dynamics in the small intestine and colon, and with transcription in the liver. These results indicate that a stable balance, between various mutagenic and DNA repair processes, is maintained throughout life and that the activity of these processes in adult stem cells varies between tissues.

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### C03.5

#### Exome-wide evaluation of splice-disrupting mutations in 4,294 families with severe developmental disorders

*J. Lord<sup>1</sup>, J. McRae<sup>1</sup>, M. Hurles<sup>2</sup>, on behalf of the Deciphering Developmental Disorders study; Wellcome Trust Sanger Institute, Hinxton, United Kingdom.*

Mutations affecting splicing are a significant contributor to human disease. Classification of splicing variants is often binary, with only those that fall within the canonical splice site (CSS) assumed to affect splicing, and to be akin to other protein-truncating variants (PTVs). We performed both population genetic and disease-burden analyses to better understand the distribution of splice-disrupting variants in and around the CSS using 4,294 exome sequenced trios from the Deciphering Developmental Disorders (DDD) study. We also used these data to compare the performance of several splicing prediction tools.

We observed that a subset of positions outside the CSS (including the 5th base after the intron/exon boundary at the donor end, and the last base of the exon) exhibited evidence of both high levels of purifying selection, and excess de novo mutations in patients. Additionally, within the two bases of canonical donor sites we observed strong evidence of discordant impact on splicing, with the base proximal to the exon showing significantly higher de novo enrichment and singleton ratio. We observed high concordance of the splicing prediction tools with our observations, however they failed to capture completely the landscape of splice-disrupting variation.

Taken together, these data suggest binary classification of variants as "splicing" only if they are in the CSS is oversimplified, and that this has a substantial impact on both the sensitivity and specificity of identifying genuine PTVs.

C03.6

## Proteome-wide expression and turnover analysis quantify genetic impact in Down Syndrome

Y. Liu<sup>1</sup>, C. Borel<sup>2</sup>, L. Li<sup>3</sup>, T. Mueller<sup>4</sup>, P. Boersema<sup>4</sup>, P. Germain<sup>5</sup>, G. Testa<sup>5</sup>, A. Beyer<sup>3</sup>, S. Antonarakis<sup>3,6</sup>, R. Aebersold<sup>1,6</sup>;

<sup>1</sup>Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland, <sup>2</sup>Department of Genetic Medicine and Development, University of Geneva Medical School, and University Hospitals of Geneva, Geneva, Switzerland, <sup>3</sup>University of Cologne, Cologne, Germany,

<sup>4</sup>Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland,

<sup>5</sup>Department of Experimental Oncology, European Institute of Oncology, Milan, Italy,

<sup>6</sup>These labs contribute equally, Zurich and Geneva, Switzerland.

**Introduction:** Trisomy 21 (T21) is the cause of Down Syndrome. However, how T21 impact human functional proteome remains unclear. We hypothesize that the proteome-wide turnover analysis is crucial to understand the functional impact of T21.

**Materials and Methods:** We investigated the effect of the extra chr21 at the levels of transcript quantity, proteome quantity and protein turnover rate. We analyzed the primary fetal skin fibroblasts derived from a pair of monozygotic twins discordant for T21, which uniquely allowed us to characterize the proteome changes due to T21 without the noise of genomic variability. To validate, we also analyzed the fibroblasts from 11 unrelated T21 individuals and 11 controls. We applied the cutting-edge SWATH mass spectrometry to reproducibly measure the proteomes.

**Results:** We quantified 4056 unique proteins for expression and ~2200 proteins by pulsed SILAC experiment for turnover analysis in both normal and T21 fibroblasts from the twins. The proteome-wide T21/normal fold-change correlation was extremely low, indicating substantial post-transcriptional regulation and buffering effects in T21. Overall, the protein degradation was faster in trisomy cells than the controls. Remarkably, those Chr21 encoded proteins that are members of heteromeric protein complexes in particular seemed to be exempt from responding to copy number alternations, likely through accelerated protein degradation. Moreover, we found that both mitochondrial and cytosolic ribosomal proteomes were degraded heavily in T21, but different degree of translational regulation shaped their final, divergent expression levels.

**Conclusion:** Prevalent, organelle specific proteome remodeling was identified as the proteomic hallmark of T21 as compared to normal.

these include 270 rare and 258 low-frequency variants (allele frequency <1% and 1-5% respectively), and 44 „high impact“ variants of large phenotypic effects. Significant enrichments of the corresponding gene sets within relevant biological pathways, rare haematological and immune disorders, mouse phenotypes, and human complex disease confirm their importance for understanding of haematopoietic processes and pathogenesis.

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C04.2

## Genome-wide association study identifies 15 novel genetic variants contributing to variation in cytokine levels

V. Kumar<sup>1</sup>, Y. Li<sup>1</sup>, M. Oosting<sup>2</sup>, I. Ricaño-Ponce<sup>1</sup>, S. Smeekens<sup>2</sup>, M. Jaeger<sup>2</sup>, L. Joosten<sup>2</sup>, C. Wijmenga<sup>1</sup>, M. Netea<sup>2</sup>;

<sup>1</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>2</sup>Radboud University Medical Center, Nijmegen, Nijmegen, Netherlands.

**Introduction:** The genetic basis of human immune responses to pathogens has a profound effect on susceptibility to human infectious and immune-mediated diseases. A highly diverse set of immune cells is involved in producing cytokines to regulate these immune responses. However, the impact of genetic variation on the production of these regulatory cytokines during the course of infection is unknown.

**Materials and methods:** In this study we profiled genome-wide SNP genotypes, FACS assessment of the cell-populations as well as cytokine responses to major human pathogens (bacteria, virus and fungus) in whole blood, PBMCs, macrophages and T-cells of 500 healthy individuals. We performed a systematic study to reveal organization of cytokine responses to different pathogens in different cell-types. We then correlated the cytokine levels to SNPs and performed integrative genomics analysis to identify genes that control cytokine production in different immune cells.

**Results and conclusions:** We have identified 15 novel loci significantly associated ( $P < 5 \times 10^{-8}$ ) with different cytokine levels in response to pathogens. We show that the cytokine-QTLs are under positive selection and are enriched to be associated with human diseases. Furthermore, SNPs affecting monocyte-derived cytokines are enriched to be associated with infectious diseases, whereas SNPs that affect T-cell derived cytokines are associated with autoimmune diseases. We show that the genetic variability in the immune pathways is organized around a physiological response to specific pathogens. In summary, we show that upon stimulation genetic variation plays a significant role in influencing human immune responses and diseases.

C04.3

## Genetic variants regulate adaptive and innate immune cell levels in the healthy Dutch population

R. Aguirre-Gamboa<sup>1</sup>, H. J. P. M. Koenen<sup>2</sup>, I. Ricano-Ponce<sup>1</sup>, R. G. van der Molen<sup>2</sup>, E. van Rijssen<sup>3</sup>, B. van Cranenbroek<sup>3</sup>, V. MagadiGopalaiyah<sup>1</sup>, C. Wijmenga<sup>1</sup>, M. Netea<sup>4</sup>, I. Joosten<sup>3</sup>, Y. Li<sup>1</sup>;

<sup>1</sup>University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands, <sup>2</sup>Radboud University Nijmegen Medical Center, Department of Internal Medicine, Nijmegen, Netherlands, <sup>3</sup>Radboud University Nijmegen Medical Center, Laboratory Medicine, Nijmegen, Netherlands, <sup>4</sup>Radboud University Nijmegen Medical Center, Department of Medicine and Nijmegen University Centre for Infectious Diseases, Nijmegen, Netherlands.

Levels of immune cell subpopulations vary greatly between healthy individuals, yet the origin of such variation is only partially known. In this study we explore the immune cell landscape for factors that influence cell abundance. We measured over 210 different absolute cell levels and cell percentages from B, NK and T cells subpopulations from fresh blood samples of 500 healthy Dutch individuals. We observed a consistent gender effect, where females have higher cell levels, an overall negative correlation with age and a considerable fluctuation with respect to season. In addition, we assessed the influence of hormones and circulating antibodies in the immune system and found no significant effect of these on cell variation. Next, we profiled the genotype of the 500 volunteers and searched for genetic associations to the absolute cell levels and proportions, these traits were controlled for gender, age effect and date of collection to evaluate for cell count QTLs (ccQTL) on ~7 million variants. We identified 11 independent loci associated with at least one cell type. Of these, 5 previously reported loci were replicated. Additionally, we performed an integrative genomics analysis to identify genes implicated to the variation of the cell levels, using RNA-seq data from blood of ~600 samples from an independent Dutch cohort, we performed cis-eQTL mapping and prioritized candidate genes for the novel loci. Furthermore, we detected that ccQTLs are enriched for SNPs associated with immune-related diseases. These results link genetic information with cellular and molecular phenotypes and explore its involvement in immune diseases.

## C04 Complex Traits

C04.1

### More than 1500 genetic variants regulate haematopoiesis in humans revealing novel genes and pathways

W. J. Astle<sup>1,2</sup>, H. Elding<sup>3</sup>, T. Jiang<sup>4</sup>, D. Allen<sup>4</sup>, D. Ruklisa<sup>1,2</sup>, H. Bouman<sup>3</sup>, F. Riveros-McKay<sup>3</sup>, A. L. Mann<sup>3</sup>, D. Mead<sup>3</sup>, M. A. Kostadima<sup>1</sup>, J. J. Lambourne<sup>1,5</sup>, S. Sivapalaratnam<sup>1,6</sup>, K. Downes<sup>1</sup>, K. Kundu<sup>1,3</sup>, L. Bomba<sup>3</sup>, J. R. Bradley<sup>1,7</sup>, L. C. Daugherty<sup>1,5</sup>, O. Delaneau<sup>8</sup>, S. Garner<sup>1,5</sup>, L. Grassi<sup>1,5</sup>, J. A. Guerrero<sup>1,5</sup>, M. Haimel<sup>1</sup>, M. Kamat<sup>1</sup>, B. Kim<sup>9</sup>, A. Mandoli<sup>9</sup>, J. Marchini<sup>10</sup>, S. Meacham<sup>1,5</sup>, K. Megy<sup>1,5</sup>, J. O'Connell<sup>10</sup>, R. Petersen<sup>1,5</sup>, N. Sharifi<sup>9</sup>, S. M. Sheard<sup>11</sup>, J. R. Staley<sup>1</sup>, S. Tuna<sup>1</sup>, M. v. d. Ent<sup>9</sup>, S. Wang<sup>9</sup>, E. Wheeler<sup>1</sup>, S. P. Wilder<sup>12</sup>, V. Iotchkova<sup>1,2</sup>, C. Moore<sup>1</sup>, J. Sambrook<sup>1,5</sup>, E. Di Angelantonio<sup>1</sup>, S. Kaptoge<sup>1</sup>, T. W. Kuipers<sup>13,14</sup>, M. Frontini<sup>1,5</sup>, J. Danesh<sup>1,3</sup>, D. J. Roberts<sup>10,15</sup>, W. H. Ouwehand<sup>1,3,16</sup>, A. S. Butterworth<sup>1</sup>, N. Soranzo<sup>3,1</sup>;

<sup>1</sup>University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>Cambridge Institute of Public Health, Cambridge, United Kingdom, <sup>3</sup>The Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>4</sup>NHS Blood and Transplant, Oxford, United Kingdom, <sup>5</sup>National Health Service (NHS) Blood and Transplant, Cambridge, United Kingdom, <sup>6</sup>Barts Health NHS Trust, London, United Kingdom, <sup>7</sup>National Institute for Health Research Cambridge Biomedical Research Centre, Cambridge, United Kingdom, <sup>8</sup>University of Geneva, Geneva, Switzerland, <sup>9</sup>Radboud University, Nijmegen, Netherlands, <sup>10</sup>University of Oxford, Oxford, United Kingdom, <sup>11</sup>UK Biobank Ltd, Stockport, United Kingdom, <sup>12</sup>European Bioinformatics Institute, Cambridge, United Kingdom, <sup>13</sup>University of Amsterdam, Amsterdam, Netherlands, <sup>14</sup>Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, <sup>15</sup>Churchill Hospital, Oxford, United Kingdom, <sup>16</sup>Addenbrooke's Hospital, Cambridge, United Kingdom.

Mature blood cells are essential for oxygen transport, haemostasis and immune response. We studied 36 haematological traits (relating to mature and immature red cells, platelets and myeloid and lymphoid white cells) in individuals of European ancestry selected from the UK Biobank, UK BiLEVE and INTERVAL cohorts. For the genetic association study, we considered a total of 173,480 individuals with phenotype and genotype data passing stringent quality control filters at 29.5 million genetic variants ( $MAF > 0.01\%$ ). Effect size estimates and their standard errors were combined across the three studies using inverse-variance weighted meta-analyses.

Overall, 182,105 variants were declared to be associated with at least one blood cell trait, represented here by a set of 1,652 distinct sentinel variants. Of these 148 corresponded to previously reported GWAS hits thereby validating almost all the blood trait associations previously reported in European samples. Associations with the remaining 1,504 variants are novel, augmenting the number of known associations by over 10-fold. Remarkably,


**C04.4**

## Capture Hi-C reveals a novel causal gene, IL20RA, in the pan-autoimmune genetic susceptibility region 6q23

A. J. McGovern<sup>1</sup>, P. Martin<sup>1</sup>, S. Schoenfelder<sup>2</sup>, P. Fraser<sup>2</sup>, S. Eyre<sup>1</sup>, G. Orozco<sup>1</sup>;

<sup>1</sup>University of Manchester, Manchester, United Kingdom, <sup>2</sup>The Babraham Institute, Cambridge, United Kingdom.

**Introduction:** The majority of complex disease associations are with single nucleotide polymorphisms (SNPs) located in non-coding enhancer regions, which may regulate transcription through long-range interactions with their target genes. The 6q23 locus, associated with several autoimmune diseases including RA, contains many intergenic SNPs situated a large distance from any gene. The aim of this work was to identify causal disease genes at the locus by studying long range chromatin interactions in human T and B cell lines.

**Materials and Methods:** Capture Hi-C (CHi-C) targeting both the disease intergenic region and all promoters within 500kb of associated SNPs was used to investigate potential interactions between RA associated variants and their functional targets. Targeted 3C-qPCR analysis in B-cell lines containing the appropriate SNP genotype was used to study genotype-specific interactions.

**Results:** CHi-C identified numerous looping interactions between restriction fragments containing intergenic SNPs and the *IL20RA* gene, upstream of *IFNGR1* and lncRNAs downstream of *TNFAIP3*. *IL20RA* and *TNFAIP3* interacted with the same lncRNAs, and with each other. 3C-qPCR showed that the risk allele of the most likely causal SNP, rs6927172, was correlated with a higher frequency of interactions with *IL20RA*. The risk allele was also associated with increased expression of *IL20RA* mRNA, and increased binding of enhancer histone marks and transcription factors in T-cells.

**Conclusions:** Complex looping interactions within the 6q23 region bring RA associated SNPs, genes and regulatory elements together. This work shows that GWAS associated SNPs cannot simply be assigned to the nearest gene, potentially identifying novel targets for new therapies.


**C04.5**

## Variants from the exome chip and metabolic pathways of type 2 diabetes

S. Jäger<sup>1,2</sup>, S. Wahl<sup>3,4</sup>, J. Kröger<sup>1,2</sup>, P. Hoffmann<sup>5,6,7</sup>, A. Floege<sup>8</sup>, T. Pischon<sup>9</sup>, J. Adamski<sup>10</sup>, M. Müller-Nurasyid<sup>1,1,2,13</sup>, M. Waldenberger<sup>3,4</sup>, K. Strauch<sup>1,1,4</sup>, A. Peters<sup>3,13,2</sup>, C. Gieger<sup>3,4</sup>, K. Suhre<sup>15</sup>, H. Grallert<sup>3,4,2</sup>, H. Boeing<sup>9</sup>, M. B. Schulze<sup>1,2</sup>, K. Meidtner<sup>1,2</sup>,

<sup>1</sup>Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany, <sup>2</sup>German Center for Diabetes Research (DZD), Neuherberg, Germany, <sup>3</sup>Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, <sup>4</sup>Research

Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, <sup>5</sup>Division of Medical Genetics, Department of Biomedicine, University of Basel, Basel, Switzerland, <sup>6</sup>Department of Genomics, Life and Brain Center, Bonn, Germany, <sup>7</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>8</sup>Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany, <sup>9</sup>Molecular Epidemiology Group, Max Delbrueck Center for Molecular Medicine (MDC), Berlin-Buch, Germany, <sup>10</sup>Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Zentrum München, Neuherberg, Germany, <sup>11</sup>Institute of Genetic Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany,

<sup>12</sup>Department of Medicine I, University Hospital Grosshadern, Ludwig-Maximilians-Universität, Munich, Germany, <sup>13</sup>DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany, <sup>14</sup>Institute of Medical Informatics, Biometry and Epidemiology, Chair of Genetic Epidemiology, Ludwig-Maximilians-Universität, Munich, Germany, <sup>15</sup>Department of Physiology and Biophysics, Weill Cornell Medical College in Qatar, Qatar Foundation-Education City, Doha, Qatar.

**Background:** The unexplained heritability of type 2 diabetes is high, suggesting that low frequency or rare genetic variants might explain additional proportions. Diabetes-related metabolites may aid the identification of novel risk variants for type 2 diabetes.

**Material and Methods:** We used the Illumina HumanExome v1.1 Bead Array to genotype individuals from the German EPIC-Potsdam study. Within a representative subsample (n=2500) we explored the association between single genetic variants and diabetes-related metabolites measured by a targeted metabolomics platform (Biocrates). Findings ( $p < 1.64 \times 10^{-7}$ ) were replicated within the German KORA F4 study. For the replicated genetic variants associations with type 2 diabetes risk were investigated within the EPIC-Potsdam case-cohort (n=2891; n<sub>cases</sub>=758) and by look-up in DIAGRAM and other consortia.

**Results:** We identified one new association between rs499974 (*MOGAT2*) and a diacyl-phosphatidylcholine ratio (PC aa C40:5/PC aa C38:5) and identified seven associations of loci which have been previously described with regard to metabolites, but found new ratios including sphingolipids: rs7412 (*APOE*) and SM (OH) C22:2/SM (OH) C22:1; rs7157785 (*SGPP1*) and SM C16:1/PC aa C28:1; SM (OH) C22:2/SM C24:0; SM (OH) C22:2/SM (OH) C14:1; SM (OH) C22:2/SM (OH) C22:1; rs364585 (*SPTLC3*) and SM C16:1/

SM C18:0; SM (OH) C22:2/SM C16:1. Previously known variants are located in *FADS1-2*, *CPS1*, *REV3L*, *NTAN1* and *PNLIPRP2*. Four replicated common variants (rs3204953, rs174550, rs499974, rs7157785) showed nominally significant associations with type 2 diabetes in DIAGRAM.

**Conclusion:** We identified eight new associations between genetic variants and diabetes-related metabolic traits providing insights into metabolic pathways underlying the development of type 2 diabetes.

**C04.6**

## StatinGWAS: A genome-wide association study demonstrating the research potential of nationwide prescription drug registries

P. Helkkula<sup>1</sup>, I. Surakka<sup>1,2</sup>, P. Häppölä<sup>1</sup>, V. Salomaa<sup>3</sup>, A. Palotie<sup>4,5</sup>, M. J. Daly<sup>6</sup>, S. Ripatti<sup>1,2,7</sup>,

<sup>1</sup>Institute for Molecular Medicine Finland, FIMM, Helsinki, Finland, <sup>2</sup>Unit of Public Health Genomics, National Institute for Health and Welfare, Helsinki, Finland, <sup>3</sup>Department of Health, National Institute for Health and Welfare, Helsinki, Finland, <sup>4</sup>Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA, United States, <sup>5</sup>The Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>6</sup>Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, United States, <sup>7</sup>Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

National prescription drug registries are a potential wealth of insight into the clinical status of large populations. While hospitalization records (e.g. ICD codes) may provide complete ascertainment of serious diseases, prescription registries may provide insights into the onset and severity of chronic disease not available solely through hospitalizations - particularly in populations with complete and relatively homogeneous access to medical care. We took this approach using the FINRISK cohort studies (N=19,678) with registry data from the Finnish social insurance prescription medicine purchase database by studying genetic associations to statin use, defined as more than two purchases of statins or ezetimibe during the follow-up period 1995-2011. Using logistic regression, we detected associations in five loci, including well-known LDL cholesterol (LDL-C) loci: LDLR (rs141787760, p=1.95e-14), PCSK9 (rs11591147, p=1.78e-14) and APOE (rs7412, p=6.13e-22). Additionally, we saw strong association to the statin target gene HMGCR (rs10045497, p=1.38e-9). Our results validate the statin use data and the drug registry for various genome-wide association study designs. Furthermore, we demonstrate that by combining a scan for LDL-C in the untreated population with the independent case-control analysis of statin use vs non-statin use, we obtain a more powerful statistical scan for LDL-C loci. Retrospective drug use data enables us to extend our study into other statin-related outcomes, such as switching medications and discontinuation for possible identification of genetic markers associated with side-effect sensitivity. We will also pursue this study approach into other drug categories, including antihypertensive drugs.

## C05 Cancer Predisposition

**C05.1**

### Germline and somatic FGFR1 abnormalities in dysembryoplastic neuroepithelial tumors

B. Rivera<sup>1</sup>, T. Gayden<sup>1</sup>, J. Carrot-Zhang<sup>1</sup>, J. Nadaf<sup>1</sup>, T. Boshart<sup>1</sup>, D. Faury<sup>1</sup>, M. Zeinieh<sup>1</sup>, R. Blanc<sup>1</sup>, D. Burk<sup>1</sup>, S. Fahimiyah<sup>1</sup>, E. Bareke<sup>1</sup>, U. Schüller<sup>2</sup>, C. Monoranu<sup>3</sup>, R. Sträter<sup>4</sup>, K. Kerf<sup>5</sup>, T. Niederstadt<sup>6</sup>, G. Kurlemann<sup>6</sup>, B. Ellezam<sup>7</sup>, Z. Michalak<sup>8</sup>, M. Thom<sup>9</sup>, P. Lockhart<sup>9</sup>, R. Leventer<sup>9</sup>, M. Ohm<sup>10</sup>, D. MacGregor<sup>11</sup>, D. Jones<sup>12</sup>, J. Karamchandani<sup>13</sup>, C. Greenwood<sup>11</sup>, A. Berghuis<sup>8</sup>, S. Bens<sup>13</sup>, R. Siebert<sup>13</sup>, M. Zakerzewska<sup>14</sup>, P. Liberski<sup>14</sup>, K. Zakerzewska<sup>15</sup>, S. Sisodiya<sup>8</sup>, W. Paulus<sup>16</sup>, S. Albrecht<sup>1</sup>, M. Hasselblatt<sup>16</sup>, N. Jabado<sup>1</sup>, W. D. Foulkes<sup>1</sup>, J. Majewski<sup>1</sup>,

<sup>1</sup>McGill University, Montreal, QC, Canada, <sup>2</sup>Ludwig-Maximilians-University, Munich, Germany, <sup>3</sup>University of Würzburg, Würzburg, Germany, <sup>4</sup>University Children's Hospital, Münster, Germany, <sup>5</sup>University Children's Hospital Münster, Münster, Germany, <sup>6</sup>University Children's Hospital, Münster, Germany, <sup>7</sup>CHU Sainte-Justine, Montreal, QC, Canada, <sup>8</sup>UCL Institute of Neurology, London, United Kingdom, <sup>9</sup>The University of Melbourne, Melbourne, Australia, <sup>10</sup>VUMC School of Medical Sciences, Amsterdam, Netherlands, <sup>11</sup>Royal Children's Hospital, Melbourne, Australia, <sup>12</sup>German Cancer Research Center (DKFZ), Heidelberg, Germany, <sup>13</sup>University of Kiel, Kiel, Germany, <sup>14</sup>University of Lodz, Lodz, Poland, <sup>15</sup>Polish Mother's Memorial Hospital Research Institute, Lodz, Poland, <sup>16</sup>University Hospital Münster, Münster, Germany.

**Introduction:** Dysembryoplastic neuroepithelial tumors (DNETs) are benign brain tumors associated with intractable, drug-resistant epilepsy. Distinguishing DNETs from other low-grade glioneuronal tumors is challenging for neuro-pathologists. We set out to identify the genetic causes of DNETs and to clarify the molecular mechanisms underlying this condition.

**Methods:** We studied a family with multinodular DNETs together with 100 sporadic tumors referred to us as DNETs. Whole-exome sequencing was performed on 46 tumors and targeted sequencing for hotspot FGFR1 mutations and BRAFp.V600E was used on the remaining samples. Blind neuropathology review, FISH, copy number variation assays and Sanger sequencing were



used to validate the findings. Supporting evidence for functional defects was obtained by in silico modelling, Flow Cytometry and  $\beta$ -galactosidase staining.

Results: We identified a novel germline FGFR1 mutation (p.R661P) and somatic activating FGFR1 mutations (p.N546K or p.K656E) in a father and his two children with DNETs. Pathology review distinguished DNETs (WHO grade I) (45%) from non-DNETs (55%). FGFR1 alterations, mainly intragenic tyrosine kinase duplication and multiple mutants in cis, characterized DNETs (58.1%) whereas FGFR1 mutations (19%) ( $p=3.698e-05$ ) and hot-spot BRAFp.V600E (22.6%) ( $p=0.00046$ ) were identified in non-DNETs. Phospho-ERK overexpression in FGFR1p.R661P and p.N546K cells support enhanced MAPK/ERK activation in this condition.

Conclusions: This study identifies constitutional and somatic FGFR1 alterations and hotspot BRAF-V600E as key events in DNETs and non-DNET tumors respectively. The integrated pathology and molecular characterization performed reveals the key role of the MAP-Kinase pathway in these seizure-prone tumours, pointing the way towards existing targeted therapies.

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## C05.2

### Germline ESR2 mutation predisposes to medullary thyroid carcinoma and causes up-regulation of RET expression

J. Smith<sup>1</sup>, M. Read<sup>2</sup>, R. Brown<sup>3</sup>, J. Hoffman<sup>4</sup>, B. Bradshaw<sup>5</sup>, C. Campbell<sup>6</sup>, T. Cole<sup>7</sup>, J. Dieguez Navas<sup>7</sup>, F. Eatock<sup>8</sup>, J. Gundara<sup>9</sup>, E. Lian<sup>10</sup>, D. McMullan<sup>5</sup>, N. V. Morgan<sup>2</sup>, L. Mulligan<sup>10</sup>, P. Morrison<sup>11</sup>, M. Robledo<sup>12,13</sup>, M. Simpson<sup>14</sup>, V. Smith<sup>2</sup>, S. Stewart<sup>4</sup>, R. Trembath<sup>15</sup>, S. Sidhu<sup>9</sup>, F. Togneri<sup>5</sup>, N. Wake<sup>1</sup>, Y. Wallis<sup>5</sup>, J. C. Watkinson<sup>2</sup>, E. R. Maher<sup>16</sup>, C. McCabe<sup>2</sup>, E. R. Woodward<sup>6,4,1</sup>;

<sup>1</sup>Centre for Rare Diseases and Personalised Medicine, University of Birmingham, Birmingham, United Kingdom, <sup>2</sup>School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, United Kingdom, <sup>3</sup>Queen Elizabeth Hospital, Queen Elizabeth Medical Centre, Birmingham, United Kingdom, <sup>4</sup>West Midlands Regional Genetics Service, Birmingham, United Kingdom, <sup>5</sup>West Midlands Regional Genetics Laboratory, Birmingham, United Kingdom, <sup>6</sup>Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre (MAHSC), Manchester, United Kingdom, <sup>7</sup>Human Biomaterials Resource Centre, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, <sup>8</sup>Department of Endocrine Surgery, Royal Victoria Hospital, Belfast, United Kingdom, <sup>9</sup>Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, Australia, <sup>10</sup>Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University, Kingston, ON, Canada, <sup>11</sup>Centre for Cancer Research and Cell Biology, Queen's University of Belfast, Belfast, United Kingdom, <sup>12</sup>Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, <sup>13</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain, <sup>14</sup>Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London, United Kingdom, <sup>15</sup>Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, United Kingdom, <sup>16</sup>Department of Medical Genetics, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, United Kingdom.

Introduction: Familial medullary thyroid cancer (MTC) and its precursor, C cell hyperplasia (CCH), is associated with germline RET mutations causing multiple endocrine neoplasia type 2. However, some rare families with apparent MTC/CCH predisposition do not have a detectable RET mutation.

Materials and Methods: To identify novel MTC/CCH predisposition genes we undertook exome resequencing studies in a family with apparent predisposition to MTC/CCH and no identifiable RET mutation.

Results: We identified a novel ESR2 frameshift mutation, c.948delT, which segregated with histological diagnosis following thyroid surgery in family members and demonstrated loss of ESR2 encoded ER $\beta$  expression in the MTC tumour. ER $\alpha$  and ER $\beta$  form heterodimers binding DNA at specific estrogen response elements (ERE) to regulate gene transcription. ER $\beta$  represses ER $\alpha$  mediated activation of the ERE and the RET promoter contains three ERE. In vitro, we showed that ESR2 c.948delT results in unopposed ER $\alpha$  mediated increased cellular proliferation, activation of the ERE and increased RET expression. In vivo, immunostaining of CCH and MTC using an anti-RET antibody demonstrated increased RET expression.

Conclusions: Together these findings identify germline ESR2 mutation as a novel cause of familial MTC/CCH and provide important insights into a novel mechanism causing increased RET expression in tumourigenesis.

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## C05.3

### Refining the clinical classification of mismatch repair gene variants

M. Genuardi<sup>1</sup>, R. Tricarico<sup>2</sup>, M. Kasela<sup>3</sup>, C. Mareni<sup>4</sup>, B. A. Thompson<sup>5</sup>, A. Drouet<sup>6</sup>, G. Gorelli<sup>7</sup>, F. Crucianelli<sup>7</sup>, L. Staderini<sup>7</sup>, V. Ingrosso<sup>7</sup>, J. Kantelinen<sup>8</sup>, L. Papi<sup>7</sup>, M. De Angioletti<sup>9</sup>, M. Berardi<sup>4</sup>, D. Turchetti<sup>8</sup>, A. Martins<sup>8</sup>, A. B. Spurdle<sup>1,10</sup>, <sup>1</sup>Catholic University, Rome, Italy, <sup>2</sup>Fox Chase Cancer Center, Philadelphia, PA, United States, <sup>3</sup>University of Helsinki, Helsinki, Finland, <sup>4</sup>Tuscan Tumor Institute, Florence, Italy, <sup>5</sup>Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, United States, <sup>6</sup>Inserm U1079, University of Rouen, Rouen, France, <sup>7</sup>University of Florence, Florence, Italy, <sup>8</sup>University of Bologna, Bologna, Italy, <sup>9</sup>I, Rouen, France, <sup>10</sup>QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Classifying DNA sequence variants to explain their clinical consequences is an integral component of clinical molecular testing. The International Society for Gastrointestinal Hereditary Tumors (InSiGHT) has developed criteria for clinical interpretation of mismatch repair (MMR) gene variants and subsequently performed a comprehensive assessment of the variants present in their database. To assess the value of the components of the InSiGHT classification system, and of additional points of evidence not considered in their approach, we have performed a systematic investigation of 24 MSH2 and MLH1 variants identified in a single institution, six of which had not been previously classified. The data used for evaluation included: population frequency, co-segregation, tumor molecular characteristics (including loss of heterozygosity), RNA analyses, and in vitro functional assays. In addition, evaluation of the variants with multiple in silico programs was performed. Overall, previous classifications of 18 variants were confirmed based on novel lines of evidence, supporting the utility of the InSiGHT approach. Among the in silico programs, PON-MMR2 and MAPP-MMR had the best overall accuracy when assessed versus classifications according to InSIGHT criteria. Since one of the major problems associated with InSIGHT classification is the need of independent replication of functional results due to lack of clinically validated in vitro assays, we propose that concordance of the two programs in predicting deleterious effects might be used as a surrogate for the functional MMR test. In sum, we propose an improved strategy for MMR gene variant interpretation, which can serve as a model for other disease genes.

## C05.4

### Chromosomal mosaicism in peripheral blood and cancer risk in Fanconi Anemia

J. Reina<sup>1,2,3</sup>, R. Pujo<sup>3,4</sup>, M. López-Sánchez<sup>1,2,5</sup>, B. Rodríguez-Santiago<sup>1,2,6</sup>, M. Aza-Carmona<sup>3,4</sup>, L. A. Pérez-Jurado<sup>1,2,3</sup>, J. Surrallés<sup>3,4</sup>,

<sup>1</sup>Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Neuroscience Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain, <sup>3</sup>Centre de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ICSIII, Barcelona, Spain, <sup>4</sup>Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, Spain, <sup>5</sup>Centre de Recerca en Epidemiologia Ambiental (CREAL), Barcelona, Spain, <sup>6</sup>qGenomics Laboratory, Esplugues, Spain.

Introduction. Clonal mosaicism for chromosomal rearrangements, detectable by SNP array of DNA from any tissue, has been associated with aging and cancer risk. Fanconi anemia (FA) is a genetic disorder characterized by congenital defects, bone marrow (BM) failure and cancer susceptibility. FA patients require strict follow-up including periodic BM testing. We have studied the prevalence of clonal mosaicism in FA patients and whether it could be an early marker for cancer.

Methods. Retrospective blood DNA samples of 129 FA patients (0-50yo) were analyzed by SNP array. Mosaic events were detected with MAD software and experimentally validated by microsatellite and MLPA analyses.

Results. We detected and validated 45 mosaic events in 14/129 patients, including 7 uniparental disomies, 15 deletions, 13 duplications, 3 complex rearrangements, 2 monosomies and 5 tri/tetrasomies. We detected 6 gains in 3q in 5 patients where 4 of them developed hematologic cancer and 3 events whose intestinal BP was within HLA loci. Compared to age-matched population controls, FA individuals had a 110-250X rate of detectable mosaicism. Cancer was more frequently diagnosed (4X) in cases with detectable mosaicism during a 0-10 year follow-up.

Conclusions. In FA, clonal mosaicism prevalence is increased and related to higher cancer risk. 3q gain, typically related to leukemia, is the most common rearrangement found in mosaicism in our cohort and in those patients with leukemia. Therefore, mosaicism detection in blood by SNP array could be used as an early marker for cancer in FA.

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**C05.5**

## Parent inheritance of RB1 hypomorphic mutations and somatic mosaicism can explain low penetrance in retinoblastoma.

V. Imperatore<sup>1</sup>, A. Pinto<sup>2,1</sup>, S. Amitrano<sup>2</sup>, S. Somma<sup>1,2</sup>, M. Bruttini<sup>1,2</sup>, T. Hadjistilianou<sup>3</sup>, S. De Francesco<sup>3</sup>, P. Toti<sup>4</sup>, E. Gussoni<sup>5</sup>, M. Mencarelli<sup>1,2</sup>, F. Mari<sup>1,2</sup>, A. Renieri<sup>1,2</sup>, F. Arian<sup>2,1</sup>;

<sup>1</sup>Medical Genetics, Department of Medical Biotechnology, University of Siena, Policlinico, Santa Maria alle Scotte, Siena, Italy, <sup>2</sup>Medical Genetics, Azienda Ospedaliera Universitaria Senese, Siena, Italy, <sup>3</sup>Unit of Ophthalmology, and Retinoblastoma Referral Center, Department of Surgery, University of Siena, Policlinico, Santa Maria alle Scotte, Siena, Italy, <sup>4</sup>Department of Human Pathology and Oncology, Section of Pathology, University of Siena, Policlinico, Santa Maria alle Scotte, Siena, Italy, <sup>5</sup>Unit of Ophthalmology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy.

Retinoblastoma (RB) represents one of the most extensively studied pediatric cancers but many questions still remain unsolved, including incomplete penetrance. In 2009, it has been demonstrated a ~3 fold excess of the RB1 maternal canonical transcript due to an imprinting mechanism. As a consequence, maternally inherited hypomorphic mutations may retain sufficient suppressor activity to prevent tumor development. Here, we change the perspective of retinoblastoma field demonstrating a differential allelic expression of hypomorphic variants that significantly influence the phenotypic outcome in families at risk for RB onset. In one family, the father who presented with retinoma and the two children who developed retinoblastoma were all identified harboring an hypomorphic splicing variant (c.2663+2T>C). RT-PCR quantitative analysis showed a higher expression of the abnormally spliced product in the father along with an inversion of the ratio between the two differentially spliced isoforms. A parent-of-origin effect was demonstrated for another hypomorphic mutation (p.Arg661Trp) able to induce RB in a child of a male carrier with an anamnestic history positive for osteosarcoma. For the first time, here we also identified somatic mosaicism as important factor that can impact the phenotypic expression. Indeed, segregation analysis performed by high-depth NGS assay in another family, showed a ~50% of mutated molecules in the proband with retinoblastoma in comparison with a ~30% in the father with retinoma, accordingly with a somatic mosaicism. These findings completely change the genetic and prenatal counselling and the clinical management of the families. They also underline the importance of performing high-sensitivity NGS for RB diagnosis.

**C05.6**

## Low-level APC mutational mosaicism is the underlying cause in a substantial fraction of unexplained colorectal adenomatous polyposis cases

I. Spier<sup>1,2</sup>, D. Drichel<sup>3</sup>, M. Kerick<sup>4,5</sup>, J. Kirlf<sup>6,2</sup>, S. Horpaapan<sup>1,7</sup>, A. Laner<sup>8,9</sup>, S. Holzapfel<sup>1,2</sup>, S. Peters<sup>1</sup>, R. Adam<sup>1,2</sup>, B. Zhao<sup>10</sup>, T. Becker<sup>3,11</sup>, R. P. Lifton<sup>10</sup>, S. Perner<sup>12,13</sup>, P. Hoffmann<sup>1,14,15</sup>, G. Kristiansen<sup>6,2</sup>, B. Timmermann<sup>16</sup>, M. M. Nöthen<sup>1,14</sup>, E. Holinski-Feder<sup>8,9</sup>, M. R. Schweiger<sup>4,5</sup>, S. Aretz<sup>1,2</sup>;

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Center for Hereditary Tumor Syndromes, University of Bonn, Bonn, Germany, <sup>3</sup>German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany, <sup>4</sup>Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>5</sup>Epigenomics, University of Cologne, Cologne, Germany, <sup>6</sup>Institute of Pathology, University of Bonn, Bonn, Germany, <sup>7</sup>Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand, <sup>8</sup>Medizinische Klinik - Campus Innenstadt, Klinikum der LMU, Munich, Germany, <sup>9</sup>MGZ - Center of Medical Genetics, Munich, Germany, <sup>10</sup>Departments of Genetics, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, United States, <sup>11</sup>Institute of Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany, <sup>12</sup>Section for Prostate Cancer Research, Institute of Pathology, Center for Integrated Oncology Cologne/Bonn, University Hospital of Bonn, Bonn, Germany, <sup>13</sup>Pathology Network of the University Hospital of Luebeck and Leibniz Research Center Borstel, Borstel, Germany, <sup>14</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany, <sup>15</sup>Division of Medical Genetics, University Hospital Basel and Department of Biomedicine, University of Basel, Basel, Switzerland, <sup>16</sup>Next Generation Sequencing Group, Max Planck Institute for Molecular Genetics, Berlin, Germany.

**Background:** In 30-50% of patients with colorectal adenomatous polyposis, no germline mutation in the known genes APC, causing familial adenomatous polyposis (FAP); MUTYH, causing MUTYH-associated polyposis (MAP); or POLE or POLD1, causing Polymerase-Proofreading-associated polyposis (PPAP) can be identified, although a hereditary etiology is likely. This study aimed to explore the impact of APC mutational mosaicism in unexplained polyposis. **Methods:** To comprehensively screen for somatic low-level APC mosaicism, high-coverage next-generation sequencing of the APC gene was performed using DNA from leukocytes and a total of 53 colorectal tumors from 20 unrelated patients with unexplained sporadic adenomatous polyposis. APC mosaicism was assumed if the same loss-of-function APC mutation was present in  $\geq$  two anatomically separated colorectal adenomas/carcinomas per patient. All mutations were validated using diverse methods. **Results:** In 25% (5/20) of patients, somatic mosaicism of a pathoge-

nic APC mutation was identified as underlying cause of the disease. In 2/5 cases, the mosaic level in leukocyte DNA was slightly below the sensitivity threshold of Sanger sequencing; while in 3/5 cases, the allelic fraction was either very low (0.1-1%) or no mutations were detectable. The majority of mosaic mutations were located outside the somatic Mutation Cluster Region of the gene. **Conclusions:** The present data indicate a high prevalence of pathogenic mosaic APC mutations below the detection thresholds of routine diagnostics in adenomatous polyposis, even if high-coverage sequencing of leukocyte DNA alone is taken into account. This has important implications for both routine work-up and strategies to identify new causative genes in this patient group.

## C06 Carrier and Newborn Screening (joint with EMPAG)

**C06.1**

### Responsible implementation of expanded carrier screening - Recommendations of the European Society of Human Genetics

L. Henneman<sup>1</sup>, P. Borry<sup>2</sup>, D. Chokoshvil<sup>3</sup>, M. C. Cornel<sup>1</sup>, C. G. Van El<sup>1</sup>, F. Forzano<sup>4</sup>, A. Hall<sup>5</sup>, H. C. Howard<sup>6</sup>, S. Janssens<sup>3</sup>, H. Kayserili<sup>7</sup>, P. Lakeman<sup>8</sup>, A. Lucassen<sup>9</sup>, S. A. Metcalfe<sup>10</sup>, L. Vidmar<sup>11</sup>, G. De Wert<sup>12</sup>, W. J. Dondorp<sup>13</sup>, B. Peterlin<sup>11</sup>;

<sup>1</sup>VU University Medical Center, Amsterdam, Netherlands, <sup>2</sup>Centre for Biomedical Ethics and Law, Leuven, Belgium, <sup>3</sup>Centre for Medical Genetics Ghent, Ghent, Belgium, <sup>4</sup>Medical Genetics Unit, Genova, Italy, <sup>5</sup>PHG Foundation, Cambridge, United Kingdom, <sup>6</sup>Centre for Research Ethics and Bioethics, Uppsala, Sweden, <sup>7</sup>Koc University School of Medicine, Istanbul, Turkey, <sup>8</sup>Academic Medical Centre, Amsterdam, Netherlands, <sup>9</sup>University of Southampton and Wessex Clinical Genetic Service, Southampton, United Kingdom, <sup>10</sup>Murdoch Childrens Research Institute and Department of Paediatrics, Melbourne, Australia, <sup>11</sup>Ljubljana University Medical Centre, Ljubljana, Slovenia, <sup>12</sup>Department of Health, Ethics & Society, Maastricht University, Maastricht, Netherlands.

Carrier screening is the detection of carrier status of recessive diseases in persons who do not have an a priori increased risk of being a carrier based on his/her personal or family history. Carrier screening aims to identify couples who have an increased risk of having an affected child to facilitate informed reproductive decision-making. Expanded carrier screening offers screening for multiple recessive disorders, facilitated by new genetic testing technologies. Expanded carrier screening panels that have been introduced to date have been advertised and offered on a commercial basis. In 2014, recommendations regarding responsible implementation of expanded carrier screening were developed by the Public and Professional Policy Committee (PPPC) of the European Society of Human Genetics (ESHG) and posted on the ESHG website (Feb-March 2015), for membership consultation. The recommendations address the challenges that expanded carrier screening might pose in the context of the lessons learnt from decades of population-based carrier screening. The final recommendations include e.g. the following: priority should be given to carrier screening panels that include (a comprehensive set of) severe childhood-onset disorders; the main focus should be on reporting sequence variants that clearly affect function; evaluation of new models of consent (e.g. 'generic consent') in this context is required; and, governments and public health authorities should adopt an active role in discussing the responsible introduction of expanded carrier screening. With these recommendations we aim to contribute to the public and professional discussion and to arrive at better clinical and laboratory practice guidelines.

**C06.2**

### Setting the scope of screening: ethical reflections on the offer of reproductive choice

G. Stapleton;

Maastricht University, Maastricht, Netherlands.

**Introduction:** International normative guidelines recommend that prenatal screening for the purpose of reproductive choice should only be offered for serious medical conditions that affect childhood. Whilst this position is widely endorsed, ethical concerns have been raised that such limitations to the scope of screening may challenge health services in maintaining a position of non-directivity with respect to couples' reproductive choices. In view of near future opportunities for whole genome sequencing with non-invasive prenatal testing this concern should be addressed. This paper examines whether the proposed limitations conflict with principles of non-directivity. **Methods:** A normative discourse analysis was performed in order to identify the aims of offering screening in line with proposed limitations. An integrated model for ethical reflection was then used to assess the consistency of each aim with respect to maintaining the non-directivity of

health services. Results: Three aims of the proposed scope were identified: to avoid suffering of the future child, to avoid suffering of the prospective parents, and to reduce the burden of care on society. All three aims conflict with the principle ethical requirement that health services should maintain a position of non-directivity with respect to couples' reproductive choices. Discussion: The aim of avoiding the suffering of prospective parents may be acceptable provided additional criteria are met relating to the wider social context of screening: i) is private access available for other screening options, ii) are services provided equitably following the outcomes of reproductive choice, and iii) is screening also offered prior to conception?

## C06.3

### Factors for successful implementation of population-based expanded carrier screening: what can we learn from existing initiatives?

**K. C. A. Holtkamp<sup>1</sup>, I. B. Mathijssen<sup>2</sup>, P. Lakeman<sup>2</sup>, M. C. Van Maarle<sup>2</sup>, W. J. Dondorp<sup>3</sup>, L. Henneman<sup>1</sup>, M. C. Cornel<sup>1</sup>;**

<sup>1</sup>VU University Medical Center, Amsterdam, Netherlands, <sup>2</sup>Academic Medical Center, Amsterdam, Netherlands, <sup>3</sup>Maastricht University, Maastricht, Netherlands.

**Background:** Carrier screening for autosomal recessive disorders aims to enhance reproductive decision making by identifying couples at a 1-in-4 risk in every pregnancy of having an affected child. Except for few countries or regions, carrier screening is not widely offered, and is mostly ancestry-based. Technological advances enable carrier screening for multiple diseases simultaneously. This allows universal screening regardless of ancestry (population-based expanded carrier screening). It is important to study how this can be successfully implemented, and what can be learned from already existing initiatives.

**Methods:** Factors associated with successful implementation were identified by: 1) a literature review, and 2) two case studies; studying experiences with carrier screening in two high-risk communities (a Dutch founder population, and the Ashkenazi Jewish population), including a survey among community members.

**Results:** Factors identified were: familiarity with (specific) genetic diseases and (the availability of) testing, high perceived benefits of screening (e.g. screening avoids much suffering), acceptance of reproductive options, perceived risk of being a carrier, and low perceived social barriers (e.g. stigmatization). In contrast to the Jewish community, the initial demand for screening in the Dutch founder population did not entirely come from the community itself. However, the large social cohesion of the community facilitated the implementation process.

**Conclusion:** In order to ensure successful implementation of population-based expanded carrier screening, effort should be made to increase knowledge about genetic diseases, create awareness and address personal benefits of screening in a non-directive way.

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## C06.4

### Advantages of expanded universal carrier screening: What is at stake?

**S. van der Hout<sup>1</sup>, K. Holtkamp<sup>2</sup>, L. Henneman<sup>2</sup>, G. De Wert<sup>1</sup>, W. Dondorp<sup>1</sup>;**

<sup>1</sup>Department of Health, Ethics and Society, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, <sup>2</sup>Department of Clinical Genetics, Section of Community Genetics, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

**Background:** Expanded universal carrier screening (EUCS) entails a twofold expansion of long-standing (preconception) screening protocols: it not only allows the simultaneous screening of a large list of diseases ('expanded'), but also refers to a pan-ethnic screening offer ('universal'). Advocates of EUCS mention three main moral advantages of this new proposition as compared with traditional (targeted and/or ancestry-based) forms of carrier screening: EUCS will (1) further enhance the autonomy of prospective parents by providing them with more information relevant for making reproductive choices; (2) provide equal access to carrier testing services; (3) reduce the risk of stigmatization. Our empirical ethics study aims to widen this account and provide a balanced picture of the potential pros and cons of EUCS.

**Methods:** Semi-structured interviews with 17 health (policy) professionals and representatives of patient organisations about their views on carrier screening including a possible EUCS-scenario.

**Results:** Stakeholders acknowledged the potential benefits of EUCS, but also expressed five main moral concerns: (1) Does EUCS respond to an urgent problem or population need? (2) Is it possible to offer couples both understandable and sufficient information about EUCS? (3) How will societal views on 'reproductive responsibility' change as a result of EUCS? (4) Is EUCS the best way to reach high-risk populations? (5) Will EUCS reinforce disability-based stigmatization?

**Conclusions:** While having the potential to overcome some moral limits inherent in traditional carrier screening, EUCS comes with moral challenges of its own. More research is needed to (further) anticipate the moral and practical implications of EUCS.

## C06.5

### Clinical utility of expanded carrier screening: reproductive behaviors of at-risk couples

**C. Ghiossi<sup>1</sup>, K. Ready<sup>2</sup>, C. Lieber<sup>2</sup>, J. D. Goldberg<sup>2</sup>, I. S. Haque<sup>2</sup>, G. A. Lazarin<sup>2</sup>, K. K. Wong<sup>2</sup>;**

<sup>1</sup>California State University Stanislaus, Turlock, CA, United States, <sup>2</sup>Counsyl, South San Francisco, CA, United States.

**Introduction:** Expanded carrier screening panels analyze dozens or hundreds of recessive disease genes for couples planning to have children, but literature on the clinical utility of screening conditions beyond professional guidelines is scarce. We surveyed at-risk couples for this purpose.

**Methods:** Patients used an expanded carrier screen for up to 110 genes. At-risk couples were those whose partners were also carriers for the same autosomal recessive diseases. We invited 465 consecutive at-risk couples to participate in an IRB-approved survey.

**Results:** 70 completed the survey; 13 that reported a family history were excluded from analysis. 41 were not pregnant at time of screening. Of those that were not pregnant, 63% indicated that they would choose IVF with PGD, prenatal diagnosis, gamete donation, adoption, or no reproduction. 27% indicated that they were not planning to alter reproductive plans. The remainder did not indicate clear plans. Participants who do not plan to pursue alternative options indicated perceived severity as a major reason.

Of pregnant participants, 43% elected fetal diagnosis. Two reported interest in testing, but miscarried before the procedure could be done. Seven did not consider the condition sufficiently severe to consider pregnancy termination. Of 7 pregnancies that underwent prenatal diagnosis, 5 were unaffected and 2 were affected. One of the affected pregnancies was terminated and one was continued.

**Conclusion:** Most at-risk couples altered reproductive planning, demonstrating clinical utility of this information. Perceived severity of the condition factored into decision making with milder diseases less likely to change planning.

## C06.6

### Genetic counseling in an oocyte donation program: knowledge, satisfaction and psychological impact of the expanded carrier screening

**J. Pla<sup>1</sup>, E. Clua<sup>2</sup>, M. Boada<sup>2</sup>, B. Coroleu<sup>2</sup>, P. N. Barri<sup>2</sup>, A. Veiga<sup>2</sup>, X. Estivill<sup>1</sup>, G. Lasheras<sup>3</sup>, A. Abulí<sup>1</sup>;**

<sup>1</sup>Unit of Medical Genomics, Department of Obstetrics, Gynaecology and Reproduction, Dexeus Women's Health, Barcelona, Spain, <sup>2</sup>Service of Reproduction Medicine, Department of Obstetrics, Gynaecology and Reproduction, Dexeus Women's Health, Barcelona, Spain, <sup>3</sup>Department of Psychiatry, Psychology and Psychosomatics, Quirón-Dexeus University Hospital, Barcelona, Spain.

NGS technologies have allowed to test the carrier status of a large number of diseases in a cost-effective manner and some reproductive centres call for the implementation of an Expanded Carrier Screening (ECS) test in oocyte donation (OD) programs. One of the main arguments against ECS in reproductive medicine is that its results may turn out to be psychosocially harmful to the donors. Hence, this study aims to assess the emotional and psychological impact of ECS in an OD program.

Our studied population consisted of 100 oocyte donors assessed in the framework of an OD program at Dexeus Women's Health in Barcelona. We evaluated participant's acquired knowledge, satisfaction with genetic counselling, perceived usefulness of ECS, and emotional and psychological impact (anxiety and depression measured by STAI and HADS-D scales) at three different moments: before pre-test genetic counselling session, after post-test genetic counselling session and after post-donation follow-up appointment. Although carriers' donors scored higher in the emotional and psychological impact questionnaires than those who were not carriers, none of the subjects had HADS-D and STAI scores considered pathologic at baseline, immediate or follow-up surveys. No correlation was found between the remaining variables and the psychological impact.

Our findings suggest that ECS results in oocyte donors do not seem to have a meaningful emotional and psychological impact and seems to be well tolerated and accepted by our participants. Genetic counselling at different stages of the clinical process is essential in order to achieve the purposes of decreasing anxiety in the gamete donors.

## C07 Genome Technology in the Clinic

### C07.1

#### Rapid screening of severely ill newborns and infants using whole genome sequencing

*B. Sikkelma-Raddatz<sup>1</sup>, C. van Diemen<sup>1</sup>, W. Kerstjens-Frederikse<sup>1</sup>, T. de Koning<sup>2</sup>, R. Sinke<sup>1</sup>, J. Jongbloed<sup>1</sup>, K. Abbott<sup>1</sup>, J. Herkert<sup>1</sup>, P. Neerincx<sup>1</sup>, G. de Vries<sup>1</sup>, M. Meems-Veldhuis<sup>1</sup>, M. Viel<sup>1</sup>, A. Schepers<sup>1</sup>, K. de Lange<sup>1</sup>, J. Dijkhuis<sup>1</sup>, J. van der Velde<sup>1</sup>, M. de Haan<sup>1</sup>, M. Swertz<sup>1</sup>, K. Bergman<sup>1</sup>, C. van Ravenswaaij-Arts<sup>1</sup>, I. van Langen<sup>1</sup>, R. Sijmons<sup>1</sup>, C. Wijmenga<sup>1</sup>;*  
<sup>1</sup>University Medical Center Groningen, Department of Genetics, Groningen, Netherlands,  
<sup>2</sup>University Medical Center Groningen, Beatrix Children's Hospital, Groningen, Netherlands.

For severely ill newborns quick molecular diagnoses are of utmost importance for clinical decision-making and can prevent unnecessary and sometimes invasive diagnostics. To date, immediate molecular testing is not a routine procedure for all patients since this is available only for few diseases. Here we present a procedure to analyze 2800 genetic disorders in severely ill newborns and infants by rapid whole-genome sequencing (WGS), and completed in approximately 2 weeks. WGS is carried out in parallel to standard diagnostic procedures. The final evaluation of the results is done by a multidisciplinary team. Thus far we have included 22 patients in the study and have provided a diagnosis of a monogenic disease for four patients. These patients presented with different clinical characteristics and that could be explained by mutations in the EIF2B5, EPG5, KLHL41 and RMND1 genes. One patient was diagnosed with a 1p36 microdeletion upon routine diagnostic testing.

In 12 patients without a diagnosis we got informed consent from the parents for further analysis in research setting. We prioritized genes using Network analysis based on gene co-expression and patient- specific Human Phenotype Ontology (HPO) terms. In addition RNA sequencing has been performed in a subset of child- parent trios to assess whether aberrant expression patterns can help interpreting possible pathogenic variants. This research follow up has led to three potential new diagnoses.

Currently we are evaluating the procedure. We will emphasize on phenotype selection, technical aspects of coverage and filtering methods.

### C07.2

#### Image analysis of patients with dysmorphic facial features boosts diagnostic yield in exome studies

*M. A. Mensah<sup>1</sup>, N. Hajjir<sup>1</sup>, N. Ehmke<sup>1</sup>, F. Alisch<sup>1</sup>, C. Ott<sup>1</sup>, R. Flöttmann<sup>1</sup>, M. Spielmann<sup>1</sup>, A. Thorwarth<sup>1</sup>, D. Korinth<sup>1</sup>, P. N. Robinson<sup>1</sup>, S. Köhler<sup>1</sup>, T. Zemojtel<sup>1</sup>, P. Lorini<sup>2</sup>, K. Dathé<sup>1</sup>, S. Döllken<sup>3</sup>, M. Schülke-Gerstenfeld<sup>1</sup>, S. Mundlos<sup>1</sup>, L. Graul-Neumann<sup>1</sup>, D. Horn<sup>1</sup>, P. M. Krawitz<sup>1</sup>;*

<sup>1</sup>Institut für Medizinische Genetik und Humangenetik, Charité - Universitätsmedizin - Berlin, Berlin, Germany, <sup>2</sup>Institut für Genetik, Medizinische Fakultät - Martin-Luther-Universität - Halle, Halle, Germany, <sup>3</sup>Medizinische Genetik Klein und Rost - München, München, Germany.

The combination of phenotype and genotype-based prioritization-strategies proved to be highly effective for detecting disease-causing mutations in high-throughput sequencing studies. However, the performance of these approaches also depends on the precision of the clinical description and requires some expert knowledge. Facial recognition technology, that detects dysmorphic features from two-dimensional photographs, holds the promise to assist in deep phenotyping of syndromic patients. Therefore, we evaluated the syndrome predictions of image analyses in a cohort of more than 100 patients with a diverse spectrum of confirmed molecular diagnoses of monogenic disorders with the Facial dysmorphology Novel Analysis (FDNA) technology. Automated facial recognition yields the correct diagnosis amongst the first ten suggested syndromes in more than two thirds of the cases and shows a high correlation with syndrome predictions that were based on expert annotated features. Hereby, we could confirm the diagnosis in cases with only subtle facial features amongst them patients with Rothmund-Thomson syndrome, Mowat-Wilson syndrome, Ectodermal Dysplasia, Fragile X syndrome, Rubinstein-Taybi syndrome and Mabry syndrome. When exome data of patients was filtered for rare sequence variants and intersected with genes that were associated with the likeliest syndromes according to image analysis, the pathogenic mutations could be identified in all cases with few false positives. Our results show that computer-assisted facial recognition is not only a promising technology that could be applied in the routine diagnostic workflow, but also a technology that allows diagnosis in cases with non-typical clinical presentation.

### C07.3

#### Detection of clinically relevant copy number variants by exome sequencing in a large cohort of genetic disorders

*J. Y. Hehir-Kwa<sup>1</sup>, R. Pfundt<sup>1</sup>, M. del Rosario<sup>1</sup>, L. E. L. M. Vissers<sup>1</sup>, M. P. Kwint<sup>1</sup>, I. M. Janssen<sup>1</sup>, N. de Leeuw<sup>1</sup>, H. G. Yntema<sup>1</sup>, M. R. Nelen<sup>1</sup>, D. Lugtenberg<sup>1</sup>, E. Kamsteeg<sup>1</sup>, N. Wieskamp<sup>1</sup>, A. P. A. Stegmann<sup>2</sup>, S. J. C. Stevens<sup>2</sup>, R. J. T. Rodenburg<sup>1</sup>, A. Simons<sup>1</sup>, A. R. Mensenkamp<sup>1</sup>, T. Rinne<sup>1</sup>, C. Gilissen<sup>1</sup>, H. Scheffer<sup>1,2</sup>, J. A. Veltman<sup>1,2</sup>;*  
<sup>1</sup>Radboudumc, Nijmegen, Netherlands, <sup>2</sup>Maastricht University Medical Centre, Maastricht, Netherlands.

Copy number variation (CNV) is a common source of genomic variation and an important genetic cause of disease. Microarray based CNV analysis has become a first tier diagnostic test for patients with intellectual disability, with a diagnostic yield of 10-20%. However, for most other genetic disorders the role of CNVs is less clear and genetic studies are generally limited to the study of single nucleotide variations (SNVs) and other small variants. With the introduction of exome and genome sequencing it is now possible to detect both SNVs and CNVs in a single test. Here, we have performed exome based CNV screening on data from 2,603 patients affected by a range of 14 different genetic disorders for which exome sequencing was performed in a diagnostic setting. Using read depth analysis we identified 131 clinically relevant CNVs ranging in size from 729bp – 8.4Mb. This results in 53 conclusive diagnoses, an overall diagnostic yield of 2%. CNVs were found exerting both dominant and recessive effects, as well as CNVs unmasking a recessive mutation that results in pathogenic compound heterozygous events. This study shows that CNVs play an important role in a broad range of genetic disorders and nicely illustrates how these CNVs can be readily detected from exome sequencing, without the need for additional genetic tests. This brings us closer to single test genomics.

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### C07.4

#### Ultra-sensitive mosaic mutation detection for clinical applications

*A. Hoischen<sup>1</sup>, M. Steehouwer<sup>1</sup>, M. Kwint<sup>1</sup>, R. Acuna Hidalgo<sup>1</sup>, E. Mersy<sup>2</sup>, C. Gilissen<sup>1</sup>, J. A. Veltman<sup>1,2</sup>, J. Shendure<sup>3</sup>, A. Gorlely<sup>4</sup>;*

<sup>1</sup>Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands, <sup>3</sup>Department of Genome Sciences, University of Washington, Seattle, WA, United States, <sup>4</sup>Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

Despite the great advances in the next generation sequencing field there is still room for improved targeted re-sequencing assays that combine high throughput with ultra-high sensitivity. We have now further optimized a single-molecule molecular inversion probe (smMIP) based targeted re-sequencing approach<sup>1</sup>. Single-molecule tracing is enabled using up to 4<sup>10</sup> (1,048,576) molecular tags. Consensus calling of respective PCR-duplicates allows correction for PCR and sequencing errors. The improved assay allows low-frequency or sub-clonal variant detection with variant levels of <0.05%. This assay provides very robust genotyping accuracy, high throughput, fast turnaround and cost-effectiveness. We anticipate that this or similar assays allow novel applications in which mutations are present in very low relative abundance in any given DNA sample with important new applications beyond cancer genetics.

Here we present first successful applications that include: 1.) Accurate determination of the fraction of mutated alleles for post-zygotic *de novo* mutations<sup>2</sup>. 2.) Detection of previously unrecognized mosaic disease causing mutations for rare clinical syndromes. 3.) Detection of known 'paternal age effect disorders' causing mutations as small clonal events in dissected testis material<sup>3</sup>. 4.) First evidence for presence or absence of parental alleles in cell free DNA from plasma of pregnant women.

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### C07.5

#### Enrichment of unamplified DNA and long-read SMRT Sequencing to unlock repeat expansion disorders

*Y. Tsai, D. Greenberg, T. A. Clark;*  
*Pacific Biosciences, Menlo Park, CA, United States.*

Nucleotide repeat expansions are a major cause of neurological and neuromuscular disease in humans, however, the nature of these genomic regions makes characterizing them extremely challenging. Accurate DNA sequencing of repeat expansions using short-read sequencing technologies is difficult, as short-read technologies often cannot read through regions of low

sequence complexity. Additionally, these short reads do not span the entire region of interest and therefore sequence assembly is required. Lastly, most target enrichment methods are reliant upon amplification which adds the additional caveat of PCR bias.

We have developed a novel, amplification-free enrichment technique that employs the CRISPR/Cas9 system for specific targeting of individual human genes. This method, in conjunction with PacBio's long reads and uniform coverage, enables sequencing of complex genomic regions that cannot be investigated with other technologies. Using human genomic DNA samples and this strategy, we have successfully targeted the loci of Huntington's Disease (HTT; CAG repeat), Fragile X (FMR1; CGG repeat), ALS (C9orf72; GGGGCC repeat), and Spinocerebellar ataxia type 10 (SCA10; variable ATCT repeat) for examination. With this data, we demonstrate the ability to isolate hundreds of individual on-target molecules in a single SMRT Cell and accurately sequence through long repeat stretches, regardless of the extreme GC-content. The method is compatible with multiplexing of multiple targets and multiple samples in a single reaction. This technique also captures native DNA molecules for sequencing, allowing for the possibility of direct detection and characterization of epigenetic signatures.

## C07.6

### Detection of AGG interruptions in FMR1 premutation females by single-molecule sequencing

*S. Ardui<sup>1</sup>, V. Race<sup>2</sup>, G. Mattheij<sup>2</sup>, J. R. Vermeesch<sup>1</sup>*

<sup>1</sup>Laboratory for Cytogenetics and Genome Research, Centre for Human Genetics, KU Leuven, Leuven, Belgium, <sup>2</sup>Laboratory for Molecular Diagnosis, Centre for Human Genetics, KU Leuven, Leuven, Belgium.

The FMR1 contains an unstable CGG repeat in the 5' untranslated region. This repeat is expanded from around 30 (normal) to a range of 55-200 repeats in female premutation carriers. Premutations occur in the population with an estimated frequency of about 1 in 200 and are at risk for developing FXTAS and POF. Furthermore, the germline transmission of the repeat is highly unstable, and therefore premutation females will often transmit a full mutation to their offspring.

The risk that a premutation female will transmit a full mutation is variable. The larger CGG repeats expand faster to full mutations. In addition, AGG triplets interrupting the CGG repeat reduce the risk for expansions. Despite its importance, AGG measurement is not yet a standard feature of FMR1 diagnostic work-up. If determined, AGG interruptions are detected by a Triplet-Primed PCR. Unfortunately, those AGG interruptions can be obscured because the normal and premutated allele camouflage each other's interruptions. Here, we explore single-molecule sequencing (Pacific Biosciences) to determine the AGG interruptions in a cohort of premutation females. We demonstrate that single-molecule sequencing correctly determines the size of both the normal and premutated allele for each female of the cohort. More interestingly, the single-molecule sequencing also allows the unambiguous separation of the normal from the premutated allele which enables the detection of the location and number of AGG interruptions for each allele. We foresee that this technology will replace current tests and has the potential to improve risk estimates allowing for improved genetic counseling. IWT 131787

## C08 Sharing and Mining Omics Data

### C08.1

#### Multi-tissue transcriptome analysis reveals disease-relevant and causal links between obesity and gene expression

*T. Tukiainen<sup>1,2</sup>, T. H. Pers<sup>2</sup>, F. Aguet<sup>2</sup>, A. Rissanen<sup>3</sup>, K. Ardlie<sup>2</sup>, C. M. Lindgren<sup>4</sup>, K. Pietiläinen<sup>5</sup>, S. Ripatti<sup>4,6,7</sup>*

<sup>1</sup>Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland, <sup>2</sup>Broad Institute of Harvard and MIT, Cambridge, MA, United States, <sup>3</sup>Obesity Research Unit, Research Programs Unit, University of Helsinki, Helsinki, Finland, <sup>4</sup>Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom, <sup>5</sup>Obesity Research Unit, Research Programs Unit, University of Helsinki, Helsinki, Finland, <sup>6</sup>Public Health, Faculty of Medicine, University of Helsinki, Helsinki, Finland, <sup>7</sup>Wellcome Trust Sanger Institute, Hinxton, Finland.

Obesity is a world-wide epidemic with severe health consequences. To elaborate the mechanisms linking obesity to its many sequelae, we investigated the association of body mass index (BMI), a common measure for adiposity, with gene expression (GE) assessed via RNA-sequencing in 44 tissue types, including the rarely accessible ones (e.g. brain and internal organs), in 450 genotyped donors from the GTEx project.

Significant (FDR<0.05) BMI-GE-associations are seen in several tissues, including expectedly adipose tissue and liver, but also skin and lung. These

obesity-associated genes are only partly shared between tissues indicating tissue-dependent pathophysiologies. Gene set enrichment analyses further reveal the plethora of BMI-related alterations and between-tissue interplay, including the previously documented downregulation of mitochondrial pathways in adipose tissue, which contrarily appear to be activated in adrenal gland. We also find a strong signature pointing to DNA damage in small intestine. Highlighting the disease-relevance of these BMI-associations, we observe an overlap between genes discovered in the BMI-GE analysis and genes implicated in genome-wide association studies (GWAS). E.g. GE-alterations in adipose tissue significantly overlap with immune-GWAS loci, underlining the role of inflammation in obesity and supporting epidemiological observations of potential link between obesity and immune system function. Further, we provide evidence, utilizing a Mendelian randomization framework, for BMI causally driving several of these associations. Comparison of BMI-associated expression changes of dozens of human tissues simultaneously provides an unprecedentedly extensive view into the pathophysiology of obesity. The pathways and causal links identified provide potential avenues for the treatment of the complications of obesity.

### C08.2

#### Identification of novel low frequency variants associated with susceptibility with a variety of cancers through the re-analysis of publicly available genome-wide association studies

*M. Guindo-Martínez<sup>1</sup>, F. Sánchez<sup>1</sup>, S. Bonàs-Guarch<sup>1</sup>, P. Cortés-Sánchez<sup>1</sup>, S. González<sup>1</sup>, C. Díaz<sup>1</sup>, R. M. Badia<sup>1,2</sup>, J. M. Mercader<sup>1</sup>, D. Torrents<sup>1,3</sup>*

<sup>1</sup>Barcelona Supercomputing Center, Barcelona, Spain, <sup>2</sup>Artificial Intelligence Research Institute, Bellaterra, Spain, <sup>3</sup>Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.

**Introduction:** The overall contribution of shared and specific genetic factors involved in cancer is still poorly understood. The reanalysis of available Genome-Wide Association Studies (GWAS) data with novel sequencing-based reference panels is a promising and cost-effective approach to unravel and to better characterize the genetic factors that give susceptibility to tumor related processes.

**Materials and Methods:** We developed GWImp-COMPSs, an integrated framework that performs imputation and association testing, to re-analyze 9 different cancer data, comprising around 80,000 subjects, through imputation with UK10K, 1000 Genomes phase 3 and Go-NL. To date, we have analyzed 9,124 samples for bladder cancer and 11,209 samples for lung cancer. **Results:** Beyond confirming and fine-mapping known *loci* for these cancers, we identified two novel *loci* associated with bladder cancer in the *ZCCHC7* gene (MAF= 0.01, OR=0.28, p=5.7e-11, regulatory region variant) and in the *GRIN2B* gene (MAF= 0.01, OR=4.77, p=9.1e-11, intron variant). For lung cancer, a novel associated *locus* was found near the *TECRL* gene (MAF=0.01, OR=3.16, p=2.4e-8).

Importantly, the *ZCCHC7* intragenic variant is highly conserved and, according to ENCODE and TRANSFAC, is a DNase I hypersensitive site with open chromatin histone marks (H3K4Me1, H3K4Me3 and H3K27Ac), and it is predicted to disrupt the transcription factor binding site for several transcription factors, including the binding of the tumor suppressor gene *BRCA1*.

**Conclusions:** By reanalyzing existing GWAS data with newer sequencing-based reference panels, we identified novel low-frequency variants associated with cancer risk. These findings, will ultimately allow the development of prognosis, diagnosis and therapeutic protocols.

### C08.3

#### Summary statistic imputation method enables conditional analysis across meta-analysis studies: Application to GIANT height associations from exome-chip & HapMap

*S. Rüeger<sup>1,2</sup>, A. R. Wood<sup>3</sup>, E. Marouli<sup>4</sup>, M. Graff<sup>5</sup>, C. Medina-Gomez<sup>6,7</sup>, K. Sin Lo<sup>8</sup>, GIANT consortium, Z. Kutalik<sup>1,2</sup>*

<sup>1</sup>Institute of Social and Preventive Medicine, University Hospital (CHUV), Lausanne, Switzerland, Lausanne, Switzerland, <sup>2</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland, <sup>3</sup>Genetics of Complex Traits, University of Exeter Medical School, University of Exeter, Exeter, United Kingdom, <sup>4</sup>William Harvey Research Institute, Queen Mary University of London, London, United Kingdom, <sup>5</sup>Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, <sup>6</sup>Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands, <sup>7</sup>Department of Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands, <sup>8</sup>Montreal Heart Institute, Montréal, QC, Canada.

Last year the GIANT consortium established 697 height-associated HapMap SNPs through a meta-analysis of ~250,000 individuals. Recently, the consortium combined exome-chip association results from 381'600 individuals to provide more insight into the role of coding regions in human stature regulation. While this new (unpublished) study revealed 99 height-asso-

ciated exome-chip variants (mapping to 74 loci) that are not present in the HapMap panel it remained unclear which variants represent truly novel association signals.

To ensure that the newly discovered height-associated exome-chip variants are independent of the 697 known height SNPs the conditional effect sizes derived from the same sample have to be calculated. To this end, the association summary statistics for exome-chip variants ought to be imputed using published HapMap association results.

We improved on existing methods for summary statistics imputation by more accurate calculation of the imputation quality, accounting for variations in available sample size, and using UK10K sequencing data for LD estimation with optimized shrinking. Our approach yielded a 10% reduction in root mean square error compared to standard methods. Variants with poor imputation quality ( $r^2 < 0.8$ ) markers, we additionally performed conditional summary statistic analysis to derive the effect of each (imputed) top exome variant conditional on all previously reported HapMap SNPs.

Three of those 45 variants were found to be independent of HapMap SNPs (SERPINA1, SNRPC, TSGA10IP).

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## C08.4

### RD-Connect: data sharing and analysis for rare disease research within the integrated platform and through GA4GH Beacon and Matchmaker Exchange

**S. Beltran**<sup>1,2</sup>, D. Piscia<sup>1,2</sup>, S. Laurie<sup>1,2</sup>, J. Protasio<sup>1,2</sup>, A. Papakonstantinou<sup>1,2</sup>, A. Cañada<sup>3,4</sup>, J. Fernández<sup>3,4</sup>, M. Thompson<sup>5</sup>, R. Kaliyaperumal<sup>5</sup>, S. Lair<sup>6</sup>, P. Sernadel<sup>7</sup>, M. Girdea<sup>8</sup>, M. Brudno<sup>9</sup>, A. Blavier<sup>6</sup>, R. Thompson<sup>9</sup>, H. Löchmuller<sup>9</sup>, M. Bellgard<sup>10</sup>, J. Paschall<sup>11</sup>, M. Roos<sup>5</sup>, P. t Hoen<sup>5</sup>, A. Valencia<sup>3,4</sup>, D. Salgado<sup>12,13</sup>, C. Béroud<sup>12,13,14</sup>, I. Gut<sup>1,2</sup>, RD-Connect Consortium; <sup>1</sup>Centro Nacional de Análisis Genómico (CNAG-CRG), Center for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain, <sup>4</sup>Instituto Nacional de Bioinformática (INB), Madrid, Spain, <sup>5</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>6</sup>Interactive Biosoftware, Rouen, France, <sup>7</sup>DETI/IEETA, University of Aveiro, Aveiro, Portugal, <sup>8</sup>Centre for Computational Medicine, Hospital for Sick Children and University of Toronto, Toronto, ON, Canada, <sup>9</sup>Institute of Genetic Medicine, MRC Centre for Neuromuscular Diseases, Newcastle University, Newcastle, United Kingdom, <sup>10</sup>Centre for Comparative Genomics, Murdoch University, Perth, Australia, <sup>11</sup>European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Cambridge, United Kingdom, <sup>12</sup>Aix-Marseille Université, Marseille, France, <sup>13</sup>Inserm, UMR\_S 910, Marseille, France, <sup>14</sup>APHM, Hôpital TIMONE Enfants, Laboratoire de Génétique Moléculaire, Marseille, France.

RD-Connect (rd-connect.eu) is an EU FP7 funded project building an integrated platform to narrow the gaps in rare disease research, where patient populations, clinical expertise and research communities are small in number and highly fragmented. The RD-Connect platform securely integrates -omics data with biosample and clinical information, providing not only a centralised data repository but also a sophisticated and user-friendly online analysis system. Raw Whole Genome, Exome or gene panel NGS data are deposited at the European Genome-phenome archive and processed by RD-Connect's standardised analysis and annotation pipeline. The corresponding clinical information is recorded in a connected PhenoTips instance using the Human Phenotype Ontology, OMIM and Orpha codes. The results are made available to authorised users through the highly configurable and efficient platform, which runs on a Hadoop cluster and uses Elasticsearch (platform.rd-connect.eu). The user-friendly interface enables filtering and prioritization of variants using the most common quality, genomic location, effect, pathogenicity and population frequency annotations. Additional tools, such as Exomiser, DiseaseCard, Alamut Functional Annotation (ALFA) and UMD Predictor (umd-predictor.eu) are integrated at several levels. RD-Connect has lit a GA4GH Beacon within the Beacon Network (www.beacon-network.org) and is actively involved in the development of the Matchmaker Exchange (www.matchmakerexchange.org) 1-sided match prototype. The latter will be useful to locate, without disclosing personal information, similar patients (by phenotype and genotype) in other databases which store unfiltered genomic data.

## C08.5

### The « genotype-first » approach and international matchmaking: An efficient approach for disease-causing gene identification in undiagnosed disorders with developmental anomalies.

**J. Thevenon**<sup>1</sup>, P. Kuentz<sup>2</sup>, A. Brue<sup>3</sup>, T. Jouan<sup>3</sup>, C. Poe<sup>2</sup>, M. Chevarin<sup>3</sup>, D. Lehalle<sup>1</sup>, A. Masurel-Paulet<sup>4</sup>, N. Jean-Marcais<sup>1</sup>, S. El Chehadeh-Djebar<sup>4</sup>, C. Philippe<sup>5</sup>, L. Lambert<sup>6</sup>, A. Roubertie<sup>7</sup>, M. Milh<sup>8</sup>, A. Jaquette<sup>9</sup>, C. Mignot<sup>8</sup>, D. Héron<sup>9</sup>, D. Geneviève<sup>10</sup>, M. Willems<sup>10</sup>, B. Delobel<sup>11</sup>, F. Prieur<sup>12</sup>, M. Gérard<sup>13</sup>, P. Vabres<sup>14</sup>, Y. Duffourd<sup>3</sup>, J. Rivière<sup>3</sup>, C. Thauvin-Robinet<sup>4</sup>, L. Faivre<sup>1</sup>;

<sup>1</sup>Centre de référence maladies rares, Dijon, France, <sup>2</sup>Laboratoire de Génétique

<sup>3</sup>moléculaire et Chromosomique, PTB, Dijon, France, <sup>4</sup>EA4271-Génétique des Anomalies du développement, Dijon, France, <sup>5</sup>Centre de référence maladies rares, Strasbourg, France, <sup>6</sup>Laboratoire de génétique Moléculaire, Vandoeuvre les Nancy, France, <sup>7</sup>Service de génétique, Vandoeuvre les Nancy, France, <sup>8</sup>Service de Neuropédiatrie, Montpellier, France, <sup>9</sup>Service de Neuropédiatrie, Marseille, France, <sup>10</sup>Service de génétique, APHP La Pitié Salpêtrière, Paris, France, <sup>11</sup>Centre de référence maladies rares, Montpellier, France, <sup>12</sup>Service de génétique, Lille, France, <sup>13</sup>Service de génétique, Saint Etienne, France, <sup>14</sup>Service de génétique, Caen, France, <sup>15</sup>Service de Dermatologie, Dijon, France.

Many families with a child having a developmental disorder are facing major delays in reaching a diagnosis. Families often have to cope with the difficulties of having a disease without a name, lacking a precise genetic counselling hampering parenthood projects and inappropriate clinical management. Whole exome sequencing (WES) offers an unprecedented tool for genetic testing without a priori hypotheses, specifically appropriated for undiagnosed patients with a developmental disorder. For patients with negative results of a diagnostic interpretation, a genotype-first approach may be fruitful in assessing whether a genotype, or variants in a candidate gene, can recurrently be responsible for clinically recognizable phenotypes. International data sharing allows the identification of similar patients with ultra-rare disorders. Here we present the results of a data sharing of candidate variants affecting putative disease-causing genes. This was proposed to families after a clinical WES performed in a regional centre in a diagnostic setting. By combining several methods for data sharing, and participating to the 'Matchmaker Exchange' initiative, new disease-causing genes were identified and could ultimately lead to a diagnosis for the families. From approximately 400 negative clinical WES, we have participated to the description of 20 new disease-causing genes for developmental disorders. Some examples will be given including: 1) candidate genes in a recognized ultra-rare disease, with no national clinical replication; 2) previously unrecognized syndromes arising as homogeneous disorders; 3) identification of recurrently mutated genes in clinically heterogeneous conditions that could be demonstrated by a sum of genetic results and clinical reports.

## C08.6

### DNA.Land: A community-wide platform to study millions of genomes-phenomes

**Y. Erlich**;

New York Genome Center/Columbia University, New York, NY, United States.

Understanding the genetic architecture of complex traits is a key challenge for personalized medicine. But obtaining the genomes and phenomes of large cohorts using traditional ascertainment methods are logically challenging and cost-prohibitive. As of today, two million individuals have already obtained genome-wide information on their DNA using Direct to Consumer (DTC) genomics companies. These individuals are usually driven by self-interest in genealogy and ancestry research. In our previous studies, we built a 13-million member family tree by crowdsourcing information from the same vibrant citizen genealogy community.

We developed a web-platform called DNA.Land (<https://DNA.Land>) where anyone can securely contribute her or his own DTC-generated genome data for research and connect phenotypes using questionnaires and his/her streams of social media information.

A critical concept of DNA.Land is reciprocity. To serve participants' curiosity in their genomes and family histories, our platform is built to efficiently offer analyses unavailable through DTC companies, including whole-genome imputation, refined ancestry inference, and kin-matching across company cohorts. We hope to work closely, trustworthily, and fruitfully with participants, to apply the platform for scientific benefit. We will discuss our efforts to collect family trees and phenotype streams using social media, while respecting individuals' preferences according to our data sharing guidelines. Our vision is that this platform will serve the human genetics-wide community to reach the massive scale of data needed to understand complex traits.

## C09 Prenatal Decision Making (joint with EMPAG)

### C09.1

#### Introduction of non-invasive prenatal testing as a first-tier screening test: A survey among Dutch midwives about their role as counselors

**L. Martin**<sup>1,2,3</sup>, J. Gitsels-van der Wal<sup>1,2,3</sup>, **L. Henneman**<sup>3,2</sup>;

<sup>1</sup>Midwifery Science, AVAG, Amsterdam, Netherlands, <sup>2</sup>EMGO Institute for Health and Care Research, Amsterdam, Netherlands, <sup>3</sup>VU University Medical Center, Amsterdam, Netherlands.

**Background:** In the Netherlands, non-invasive prenatal testing (NIPT) is currently only available for women at high-risk for fetal aneuploidy based on the first-trimester combined test (FCT,  $>1:200$ ) or medical history. Should NIPT become available as a first-tier screening test, the role of midwives will become even more important as 80% of pregnant women are counselled in primary care. We assessed midwives' attitudes, knowledge and educational needs regarding NIPT.

**Methods:** Online survey completed by 436 (of the 2106) Dutch midwives, April-June 2015.

**Results:** The majority (68%) of midwives were in favour of replacing FCT by NIPT, although 40% preferred to maintain nuchal translucency measurement. Only 2% preferred to implement NIPT after FCT with a lower risk cut-off ( $>1:1000$ ). Midwives were worried that women would not want to (74%) and/or cannot (65%) pay the estimated costs of NIPT (~530 euros). In total, 63% had followed training on NIPT. Overall knowledge was good, although 52% did not know fetal DNA originates from the placenta, and 35% did not know an increased (NT) is no indication for NIPT. Most midwives (87%) felt confident about counseling for NIPT; 60% believed counseling will become easier.

**Conclusions:** Midwives' attitudes towards NIPT as first-tier screening test are positive, but many prefer to maintain NT measurement. Midwives worry that many women will not or cannot pay for NIPT which may lead to inequality. Knowledge seems good but can be improved in some aspects. Education should continue to focus on counseling competences in order to facilitate women's informed reproductive decision-making.

## C09.2

### Should we be worried about children born after PGD for Huntington's Disease?

*M. den Heijer<sup>1</sup>, A. Tibben<sup>2</sup>, G. de Wert<sup>3</sup>, W. Dondorp<sup>3</sup>, M. van der Sangen<sup>3</sup>, C. de Die<sup>3</sup>;*

*<sup>1</sup>Erasmus MC, Rotterdam, Netherlands, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>Maastricht University Medical Centre, Maastricht, Netherlands.*

**Introduction:** In the Netherlands, there is broad societal and political support for preimplantation genetic diagnosis (PGD) if one of the parents is a carrier of HD. However, policymakers and funders expressed concerns about facilitating reproductive options that lead to children who will inevitably be confronted with a parent with HD, and grow up in potential distressing circumstances with consequently impairment of the quality of their life. To meet those concerns, we examined psychological functioning, coping and dynamics in these families.

**Methods:** We conducted 10 semi-structured interviews with 7 couples and 3 single parents who gave birth to at least one child after PGD. None of the carriers were symptomatic at the time of testing (1994 - 2011). At the time of the interview, 2 parents were symptomatic, one was deceased. Interviews were audio-taped, transcribed verbatim and analyzed thematically.

**Results:** PGD met parents' expectations regarding relief from guilt feelings towards their child(ren). Those who had previous prenatal diagnosis reported that termination of pregnancy was extremely psychologically burdening, whereas for PGD the procedure was more physically distressing. As long as the carrier-parent was asymptomatic, the couple's focus was mainly on the individual future disease process. When the carrier-parent becomes symptomatic, the family becomes insidiously aware of the far-reaching impact on all involved, which may initially have been underestimated.

**Conclusions:** We did not observe specific issues related to PGD. However, it is of uttermost importance that parents have access to specific health care supporting the family system in issues related to (future) HD.

## C09.3

### Informed choice in prenatal genetic testing: the choice between non-invasive and invasive prenatal testing

*S. L. van der Steen<sup>1</sup>, K. E. M. Diderich<sup>1</sup>, I. M. Bakkeren<sup>1</sup>, M. M. F. C. Knapen<sup>1</sup>, A. T. J. I. Go<sup>1</sup>, A. Tibben<sup>2</sup>, M. I. Srebnik<sup>1</sup>, D. Van Opstal<sup>1</sup>, M. G. Polak<sup>3</sup>, R. J. H. Galjaard<sup>1</sup>, S. R. Riedijk<sup>1</sup>;*

*<sup>1</sup>Erasmus University Medical Center, Rotterdam, Netherlands, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>Erasmus University, Rotterdam, Netherlands.*

**Background and aims:** In case of an increased risk at first trimester screening, most centers offer further testing by non-invasive prenatal testing (NIPT), or invasive prenatal diagnosis (PND) with rapid aneuploidy detection. In contrast, we offer whole genome SNP array analysis at 0.5 Mb resolution, which has a broader scope. We aimed to investigate whether pregnant women and their partners made informed choices between NIPT and array testing after routine pre-test counselling.

**Methods:** 330 women and 103 partners participated. Informed choice was defined as a behaviorally implemented choice that is attitude-consistent and based on sufficient knowledge, measured with the Measure of Informed

Choice. Three categories of informed decision-making were formed, 1) completely informed, 2) partly informed and 3) completely uninformed.

**Results:** 87% chose NIPT, 12% chose invasive PND. Twenty-six percent of the NIPT group made completely informed choices, versus 85% of the participants who opted for PND. In the NIPT group, 60% of choices were based on sufficient knowledge, but were inconsistent with attitude, versus 32% in the PND group. Scrutinizing the attitudes, we found that 25% of participants choosing NIPT did prefer broader testing, but were held back by risks of an invasive procedure.

**Conclusions:** Most women and their partners highly valued the avoidance of a miscarriage risk over invasive PND. Their attitudes indicate they would prefer results comparable to whole genome testing, but safely, i.e. performed by NIPT. For informed decision-making during counselling, we advocate more focus on attitude-consistency as compared to only a strong focus on knowledge.

## C09.4

### Attitudes, decision-making and experiences of preimplantation genetic diagnosis (PGD) users

*S. Zuckerman<sup>1</sup>, S. Gooldin<sup>2</sup>, G. Altarescu<sup>1</sup>;*

*<sup>1</sup>Hebrew University, Shaare Zedek Medical Center, Jerusalem, Israel, <sup>2</sup>Hebrew University, Jerusalem, Israel.*

PGD is an accepted procedure for prenatal diagnosis (PND), worldwide. The psychosocial aspects of the technology has been discussed among experts and were evaluated among various population groups but only paucity of empirical data focused on PGD users' expectations, concerns, decision-making and experiences, involved in the process.

In order to evaluate Israeli PGD users' attitudes we used mixed methods: Semi-structured in-depth interviews of 37 users (carriers of various genetic disorders) were performed. Fifty seven questionnaires, based on themes emerged in the interviews, were filled by people in various stages of PGD procedure.

Seventy nine subjects (84%) consider performing PND for a transmissible genetic disorder, yet 43 (46%) were not sure PGD should be the first choice. Once being involved in the procedure, 71 people (76%) thought PGD is the best option comparing to CVS and amniocentesis, and 54 (57%) view PGD as the preferred option for future pregnancies. Interestingly, all others adopted to avoid PND or would perform CVS, but not amniocentesis.

PGD users viewed as advantages: the reassurance of the unaffected embryo status from the beginning of pregnancy (65%), avoiding pregnancy termination (65%) and invasive prenatal tests (29%). The disadvantages included the medical actions involved (57%), the need for hormonal treatment (56%) and long waiting lists for the procedure (42%).

Understanding attitudes and experiences of couples who already performed PGD will deepen the insights of the special needs of this particular group, and should play a crucial role in developing programs for counseling, directing and accompanying future PGD users.

## C09.5

### What do pregnant women think of prenatal whole-exome sequencing?

#### A cross-cultural comparison

*C. Richards<sup>1</sup>, S. Dheensa<sup>2</sup>, A. Newson<sup>2</sup>, Z. Deans<sup>3</sup>, S. Shkedi-Rafid<sup>4</sup>, J. Hyett<sup>2</sup>, Z. Richmond<sup>2</sup>, A. Fenwick<sup>1</sup>;*

*<sup>1</sup>University of Southampton, Southampton, United Kingdom, <sup>2</sup>The University of Sydney, Sydney, Australia, <sup>3</sup>University of Bristol, Bristol, United Kingdom, <sup>4</sup>Hebrew University of Jerusalem, Jerusalem, Israel.*

**Introduction:** Prenatal whole-exome sequencing (PWES) is suggested to have a greater diagnostic yield than chromosomal-microarray analysis for diagnosing fetal anomalies. Despite falling costs and increasing speeds, clinical practice has not incorporated PWES because its clinical validity is disputed and it produces a huge range of results, some of which could influence decisions to terminate (potentially healthy) pregnancies. As almost no research explores what pregnant women think about PWES, we are comparing opinions between the UK, Australia, and Israel.

**Methods:** We created a questionnaire by first developing a pool of questions from the literature about genome tests; refined questions and thus content validity through discussion with experts; and conducted cognitive interviewing with pregnant women to increase the questionnaire's sensitivity, reliability, and validity. Piloting ( $n \approx 150$ ) and administration of the final questionnaire ( $n \approx 600$ ) will soon be underway across the three countries.

**Results:** We are illuminating views about what 'incidental findings' should be reported, when, and why; the meaning of variants of uncertain significance and whether they are grounds to terminate pregnancies; the accep-

tability and feasibility of trio or family-sequencing during pregnancy to clarify uncertain results; and acceptable turnaround times for results and who should communicate them.

**Conclusions:** We will contribute to international debates about whether clinical care should integrate PWES and highlight what women need from genetic counselling to make informed decisions. Results will also provide the first step in identifying cultural norms, ethical frameworks, and political landscapes that could affect whether and how different countries translate this technology into clinical care.

## C09.6

### Why do pregnant women accept or decline prenatal diagnosis for Down syndrome?

*C. Ingvoldstad<sup>1</sup>, E. Ternby<sup>2</sup>, O. Axelsson<sup>2,3</sup>, G. Anerén<sup>4</sup>, P. Lindgren<sup>1</sup>;*

<sup>1</sup>Dept of Clinical Science, Intervention and Technology, Division of Obstetrics and Gynaecology, Stockholm, Sweden, <sup>2</sup>Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden, <sup>3</sup>Centre for Clinical Research Sörmland, Uppsala University, Eskilstuna, Sweden, <sup>4</sup>Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, Uppsala, Sweden.

**Objective:** To investigate if knowledge of Down syndrome (DS), influences the decision to accept or decline prenatal diagnosis (PND). Secondary aims were to elucidate reasons for accepting or declining PND and investigate differences between the accepting and declining group in perceived information, knowing someone with DS and thoughts about decision-making.

**Method:** A questionnaire was completed by 76 pregnant women who underwent invasive testing and 65 women who declined tests for chromosomal aberrations.

**Results:** Apart from one question no significant differences were found in knowledge of DS between women declining or accepting PND for DS. Both groups had varying and in several aspects low levels of knowledge about DS and its consequences. Most common reasons to accept PND were 'to ease my worries' and 'to do all possible tests to make sure the baby is healthy'. Corresponding statements declining PND were 'termination of pregnancy is not an option' and 'because invasive tests increase the risk of miscarriage'. More women declining PND knew someone with DS.

**Conclusion:** Knowledge of DS at these levels is not a major factor when women decide to accept or decline PND for DS. Their choice is mostly based on opinions and moral values.

## C10 Epigenetic mechanisms in development and disease

### C10.1

#### Evolving skeletal traits by cis-regulatory changes in bone morphogenetic proteins

*V. Indjeian;*

*MRC Clinical Sciences Centre, London, United Kingdom.*

Changes in bone size and shape are defining features of many vertebrates, and underlie many of the traits that make us uniquely human. To uncover the genomic changes that control species-specific skeletal alterations, we did high-resolution mapping of dermal bone traits in sticklebacks. We identified the gene for a secreted bone morphogenetic protein, *Growth/Differentiation Factor 6* (*GDF6*), as a major locus controlling flat bone size in natural populations. Freshwater fish have a *cis*-acting regulatory change that increases *GDF6* expression, and transgenic overexpression phenocopies evolutionary changes in dermal bone size. Comparative genomics revealed that the human *GDF6* locus also has undergone distinctive regulatory evolution, including complete loss of an enhancer that is highly conserved in other mammals. Functional tests show that the ancestral enhancer drives expression in hindlimbs but not forelimbs, in locations that have been specifically modified during the human transition to bipedalism. These results add to growing evidence that *cis*-regulatory modifications of BMP genes represent a common mechanism for evolving specific skeletal changes in humans and other vertebrates.

### C10.2

#### Genetic and functional interactions between SOX10, ZEB2 and EDN3/EDNRB during mouse enteric nervous system development

*Y. Watanabe<sup>1,2</sup>, L. Stanchina<sup>1,3</sup>, L. Lecerf<sup>1,2</sup>, V. Baral<sup>1,2</sup>, V. Pingault<sup>1,4</sup>, D. Huylebroeck<sup>5</sup>, N. Bondurand<sup>1,2</sup>;*

<sup>1</sup>INSERM U955 IMRB Team 6, Créteil, France, <sup>2</sup>Université Paris Est, Faculté de Médecine,

<sup>3</sup>Créteil, France, <sup>4</sup>current address : Centre d'Etude des Cellules Souches (CECS), I-Stem, AFM, Evry, France, <sup>5</sup>current address : Institut Imagine, INSERM UMR 1163 Embryologie et génétique des malformations, Paris, France, <sup>6</sup>Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands.

Hirschsprung disease (HSCR) is characterized by the absence of enteric ganglia along a variable portion of the distal gastrointestinal tract, which results in functional intestinal obstruction. This rare developmental disorder (1/5,000 live births) is attributed to a failure of the neural crest cells (NCC) to migrate, proliferate, differentiate or survive and to form the enteric nervous system (ENS). Isolated or syndromic forms of HSCR have a complex genetic etiology with a dozen factors involved, including EDN3, its receptor EDNRB and the transcription factors SOX10 and ZEB2.

Over the last ten years, we have studied the role of these factors during ENS development and characterized the genetic and functional interactions among them. We showed that genetic interactions between Sox10 and Zeb2 or Edn3/EdnrB are essential for enteric NCC survival and differentiation. However, the cellular and molecular contribution of Zeb2 remains unclear. Here, the generation and characterization of the double-mutant mice Zeb2-Edn3 and Zeb2-EdnrB revealed a genetic interaction between these three genes in ENS development. Indeed, double-mutants present with increased distal aganglionosis as compared to single mutants. In parallel, gene regulation studies combined with enteric progenitor culture and rescue experiments allowed us to demonstrate the transcriptional regulation of EDNRB by SOX10 and ZEB2 and the essential role of the SOX10/ZEB2/EDN3 "triade" in controlling the differentiation status of enteric NCC. With these results, we can now integrate ZEB2 in the genetic interaction network known to be involved in ENS development, leading to a better understanding of the cellular bases of HSCR.

### C10.3

#### Knockout of the zinc finger protein ZNF274 in PWS-specific stem cell neurons activates expression of repressed maternal 15q11.2-q13 transcripts

*M. Langouet;*

*UConn Health, Farmington, CT, United States.*

Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia and failure to thrive during infancy, and, subsequently, by obesity, hyperphagia, cognitive disability and behavioral abnormalities. This neurobehavioral disorder of genomic imprinting is caused by the lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. The detailed genetic basis of PWS is complex involving the loss of active copies of a cluster of non-coding box C/D class small nucleolar RNAs, the SNORD116 snoRNAs. In stem cell models of PWS, we previously identified a new epigenetic complex, composed of the zinc-finger protein ZNF274 and the SET domain, bifurcated 1 (SETDB1) histone H3 lysine 9 (H3K9) methyltransferase, that is involved in silencing the maternal copy of the PWS critical region (PWS-CR) encompassing the SNORD116 cluster.

Here, using genome editing approaches, we have knocked out ZNF274 expression in PWS-specific iPSC lines from multiple PWS patients with diverse genetic abnormalities and showed that the extent of transcriptional re-activation of PWS-CR genes varies during *in vitro* neurogenesis. While PWS-CR gene expression in ZNF274 knockout (KO) iPSCs is ~5 % of normal levels, wild-type mRNA levels are completely restored in neurons derived from PWS ZNF274 KO iPSCs. Surprisingly, the DNA methylation at the PWS-IC remains unchanged upon ZNF274 KO, suggesting that the ZNF274 complex acts as a second imprinting mechanism regulating the PWS-CR gene expression during the course of neurogenesis. Our data further support the finding that ZNF274 may represent a potential target for future therapeutic applications to rescue the PWS phenotype.

### C10.4

#### Features of FMR1 hypermethylation and CGG instability in FXS pluripotent stem cells

*H. Mor-Shaked<sup>1</sup>, M. Avitzour<sup>1</sup>, G. Altarescu<sup>2</sup>, T. geva-eldar<sup>2</sup>, S. Epsztajn-Litman<sup>1</sup>, R. Eiges<sup>1</sup>;*

<sup>1</sup>Shaare Zedek Medical Center affiliated with the Hebrew University School of Medicine, jerusalem, Israel, <sup>2</sup>Shaare Zedek Medical Center, jerusalem, Israel.

Our research focuses on when and how FMR1 gains aberrant methylation, and whether hypermethylation is mechanistically associated with CGG somatic instability. Taking advantage of a large set of FXS HESC lines (9 in total), we show that *FMR1* epigenetic gene silencing commonly occurs in the undifferentiated cells and that FXS HESC lines are heterogeneous for repeat size and methylation levels. By monitoring single cell lineages of FXS HESCs and patient-derived iPSCs over time in culture, we demonstrate that CGG instability in fact exists in these cells, and is largely correlated with



transcriptional activity of FMR1. Furthermore, we provide evidence for the existence of R-Loops near the CGG repeats in FXS HESCs/IPS with an active FMR1, and demonstrate their co-existence with single strand DNA slip-outs from the G-rich non-template DNA. Taken together, we propose that the variability in methylation levels within undifferentiated FXS HESC lines reflects a widespread event within mutant FMR1 embryos, where methylation state is initially set. In accordance with our findings, we put forward a model that relies on R-loop formation to explain how epigenetics and gene transcription are mechanistically associated with CGG somatic instability in FXS.

#### C10.5

##### Fractal nature of chromatin modules of the human genome

**K. Popadin<sup>1</sup>, O. Delaneau<sup>2</sup>, M. Zazhytska<sup>1</sup>, S. Kumar<sup>3,4</sup>, A. Giovanna<sup>3,4</sup>, A. R. Gschwind<sup>1</sup>, C. Borel<sup>2</sup>, D. Marbach<sup>5</sup>, D. Lamparter<sup>6</sup>, P. Bucher<sup>3,4</sup>, S. E. Antonarakis<sup>2</sup>, E. T. Dermitzakis<sup>2</sup>, A. Reymond<sup>1</sup>:**

<sup>1</sup>University of Lausanne, Center for Integrative Genomics, Lausanne, Switzerland, <sup>2</sup>University of Geneva, Department of Genetic Medicine and Development, Geneva, Switzerland, <sup>3</sup>Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne, Switzerland, <sup>4</sup>Swiss Institute of Bioinformatics (SIB), EPFL, Lausanne, Switzerland, <sup>5</sup>University of Lausanne, Faculty of Biology and Medicine, Department of Computational Biology, Lausanne, Switzerland.

It has been recently shown that the chromatin landscape of the human genome is organized into non-uniformly distributed chromatin modules - regions of coordinated behavior of multiple chromatin marks. These modules may represent either active elements of regulatory transcription units or their passive chromatin footprints. To describe the structures of these units, find the patterns of their location, decipher the main factors driving these coordinated behaviors and ultimately reveal their functions, we need to be able to define and reconstruct these modules with high precision. Towards this we have annotated genome-wide inter-individual variation in several chromatin modifications representing active marks (i.e. enrichment of H3K27ac histone modifications), promoter marks (H3K4me3), enhancer marks (H3K4me1), genetic polymorphisms (>7.5 SNPs) and expression level (RNA-seq) in lymphoblastoid cell lines of 200 individuals. Using this high resolution data-set we discovered a fractal structure of the chromatin interactions. To uncover main factors and mechanisms maintaining these chromatin interactions along the human genome we analyzed their distribution upon stratification for evolutionary (i.e. density and properties of genes involved in the interactions), topological (i.e. variation in DNA topology between different cell lines and species) and replication (i.e. variation in replication timing) constraints. We consider the fractal network of the chromatin interactions as a meta-phenotype which can explain better the links between genotype and higher level phenotypes such as transcriptome variation.

#### C10.6

##### Hotspots of aberrant enhancer activity punctuate the colorectal cancer epigenome

**P. Scacheri<sup>1,2</sup>, A. Cohen<sup>1</sup>, O. Corradin<sup>1</sup>, J. Luppino<sup>1</sup>, C. Bartels<sup>1</sup>, J. Morrow<sup>1</sup>, S. Mack<sup>2</sup>, G. Dhillon<sup>1</sup>, M. Kalady<sup>2</sup>, J. Willis<sup>1</sup>, R. Keri<sup>1</sup>, N. Berger<sup>1</sup>, S. Markowitz<sup>1</sup>:**

<sup>1</sup>Case Western Reserve University, Cleveland, OH, United States, <sup>2</sup>Cleveland Clinic, Cleveland, OH, United States.

In addition to somatic mutations in genes, aberrant enhancer activity at non-coding regions of the genome is a key driver of tumor formation across cancer types. Through ChIP-seq of the characteristic enhancer histone marks, H3K4me1 and H3K27ac, we mapped putative gene enhancer elements across the epigenomes of a cohort of more than forty colorectal cancer (CRC) cell lines, primary CRC tumors, and normal colon epithelium samples, representing the most comprehensive characterization of enhancer elements in a single type of cancer to date. We identified sets of enhancers that are recurrently commissioned and decommissioned in CRC, including 75 and 67, respectively, present in every CRC sample analyzed, regardless of clinical stage. Multiple lines of evidence support the pathological relevance of the recurrently altered enhancers, particularly those that acquired enhancer activity. Many activate known and novel oncogenes and most are constituents of super enhancers. Nearly half of all GWAS CRC risk loci identified to date map to the recurrently acquired enhancers. CRC growth can be mitigated through pharmacologic inhibition or targeted knockout of genes activated by the recurrent enhancers. The majority of the recurrently acquired enhancers originate from primed chromatin, suggesting that activation of primed chromatin is the mechanism for their formation. Overall, these findings indicate that the CRC epigenome is defined by highly recurrent epigenetic alterations at enhancer elements, or "hotspots". These hotspots activate a common aberrant transcriptional program necessary for CRC tumor growth and survival, and therefore represent promising targets for anti-cancer therapies.

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#### C11.1

##### Deciphering phenotypic variability of genomic disorders using the 16p11.2 syndromes as a paradigm

**K. Männik<sup>1,2</sup>, A. M. Maillard<sup>3</sup>, B. Kolk<sup>2,4</sup>, K. Popadin<sup>1</sup>, S. Pradervand<sup>1,5</sup>, M. Kals<sup>2,6</sup>, B. Rodriguez-Herreros<sup>3,7</sup>, A. Pain<sup>3</sup>, S. Martin-Bretet<sup>3</sup>, J. Kosmicki<sup>8,9</sup>, S. Lebon<sup>10</sup>, M. Noukas<sup>2,4</sup>, 16p11.2 European Consortium, Simons VIP Consortium, M. M. van Haelst<sup>11</sup>, A. Metspalu<sup>2,4</sup>, N. Katsanis<sup>12</sup>, M. J. Daly<sup>8,9</sup>, J. S. Beckmann<sup>5,3</sup>, S. Jacquemont<sup>1,13</sup>, A. Reymond<sup>1</sup>:**

<sup>1</sup>Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland,

<sup>2</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>3</sup>Service of Medical Genetics, Lausanne University Hospital, Lausanne, Switzerland, <sup>4</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia, <sup>5</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland, <sup>6</sup>Institute of Mathematics and Statistics, University of Tartu, Tartu, Estonia, <sup>7</sup>Laboratory for Research in Neuroimaging, Department of Clinical Neurosciences, Lausanne University Hospital, Lausanne, Switzerland, <sup>8</sup>Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, <sup>9</sup>Program in Medical and Population Genetics and Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA, United States, <sup>10</sup>Pediatric Neurology Unit, Department of Pediatrics, Lausanne University Hospital, Lausanne, Switzerland, <sup>11</sup>Department of Genetics, University Medical Centre Utrecht, Utrecht, Netherlands, <sup>12</sup>Center for Human Disease Modeling and Department of Cell Biology, Duke University, Durham, NC, United States, <sup>13</sup>CHU Sainte-Justine Research Centre, Montreal, QC, Canada.

Reciprocal 16p11.2 CNVs are characterized by considerable variance in their impact on cognition, behavior, head size and BMI suggesting that yet unidentified modifying factors may contribute to the patients' phenotypic outcome. Our recent findings indicated a possible contribution of ciliary dysfunction to the phenotypes of the 16p11.2 CNVs. To potentially identify driver and modifier genes of the 16p11.2 endophenotypes we have sequenced the exomes and the transcriptomes of 250 deeply phenotyped individuals from 16p11.2 families. To avoid ascertainment bias and better represent the phenotypic spectrum, we recruited carriers among unselected population cohorts, as well as in clinical settings. We are cataloging potentially deleterious variants in the 16p11.2-altered pathways, and performing correlative analyses between quantitative traits (e.g. BMI, IQ) and transcript levels. Our results will be replicated with 306 exomes of 16p11.2 trios from the Simons VIP Consortium.

The pilot phase of data analysis identified slightly deleterious variants in 16p11.2-interacting genes that potentially contribute to vertebral and Müllerian duct defects recurrently seen in a subset of 16p11.2 patients. We have also identified, for example, an individual carrying a 16p11.2 deletion and a heterozygous loss-of-function variant in the pan-ciliopathy gene CEP290, and an individual with a 16p11.2 duplication and a non-synonymous mutation in the rasopathy gene PTPN11. Compatible with our "two-hit" hypothesis these patients present alleviated and aggravated phenotype spectra, respectively. Our study will have potential implications for precise clinical management of 16p11.2 CNV carriers, and will shed light to the mechanisms contributing to the complex etiologies of genomic disorders.

#### C11.2

##### The power of protective modifiers in human genetics: Plastin3 and Coronin1C unravel endocytosis as an essential cellular mechanism disturbed in spinal muscular atrophy

**L. Torres Benito<sup>1,2,3</sup>, S. M. Hosseinibarkooie<sup>1,2,3</sup>, M. Peters<sup>1,2,3</sup>, R. Rastetter<sup>4</sup>, K.**

**Hupperich<sup>1,2,3</sup>, A. Hoffmann<sup>1,2,3</sup>, N. Mendoza Ferreira<sup>1,2,3</sup>, A. Kaczmarek<sup>1,2,3</sup>, E. Janzen<sup>1,2,3</sup>, T. Lamkaemeyer<sup>5</sup>, C. Guschlbauer<sup>6</sup>, A. Büschges<sup>7</sup>, M. Hammerschmidt<sup>2,5,8</sup>, M. Riessland<sup>1,2,3</sup>, M. J. Kye<sup>1,2,3</sup>, C. Clemen<sup>9</sup>, B. Wirth<sup>1,2,3</sup>:**

<sup>1</sup>Institute of Human Genetics, Cologne, Germany, <sup>2</sup>Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany, <sup>3</sup>Institute for Genetics, University of Cologne, Cologne, Germany, <sup>4</sup>Center for Biochemistry, Institute of Biochemistry, Cologne, Germany, <sup>5</sup>Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), Cologne, Germany, <sup>6</sup>Biocenter, Institute for Zoology University of Cologne, Cologne, Germany, <sup>7</sup>Biocenter Cologne, Institute for Zoology, University of Cologne, Cologne, Germany, <sup>8</sup>Institute for Developmental Biology, University of Cologne, Cologne, Germany, <sup>9</sup>Center for Biochemistry, Institute of Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany.

Paradoxically, mutations in some ubiquitously expressed housekeeping genes impair only a specific cell type. A particularly remarkable example is spinal muscular atrophy (SMA), one of the most common autosomal recessive disorders in humans. Twenty years after SMN1 has been discovered as the SMA determining gene, a major unsolved question is still which cellular mechanism leads to motoneuron dysfunction and ultimately to SMA. Here we demonstrate the power of the protective genetic modifier, plastin 3 (PLS3) to answer this question. Homozygous loss of SMN1 causes SMA. SMN2 produces ~10% functional SMN protein, insufficient to counteract

SMA. In contrast, elevated PLS3, an F-actin -bundling protein fully protects against SMA in SMN1-deleted individuals, carrying 3-4 SMN2 copies. We prove here that PLS3 rescued survival (>250 days) and motoric abilities in an intermediate SMA mouse model. Since PLS3 knock-out in yeast impairs endocytosis, we hypothesized that disturbed endocytosis might be a key cellular mechanism underlying impaired neurotransmission and NMJ maintenance in SMA. Indeed, SMN deficit dramatically reduced endocytosis, which was restored to normal levels by PLS3 overexpression. Under low frequency electro-stimulation, endocytic FM-143 uptake at NMJ level was restored to control levels in SMA-PLS3 as compared to SMA mice. Moreover, proteomics and biochemical analysis unraveled CORO1C, another F-actin binding protein, to directly bind to PLS3 in a calcium-dependent manner. As PLS3, CORO1C overexpression restored fluid-phase endocytosis in SMN knockdown cells by elevating F-actin levels and rescued the axonal truncation and branching phenotype in Smn-depleted fish.

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### C11.3

#### Mutations in the ICF gene DNMT3B modify the epigenetic repression of the D4Z4 repeat and the penetrance of facioscapulohumeral dystrophy

*M. L. van den Boogaard<sup>1</sup>, R. J. L. F. Lemmers<sup>1</sup>, J. Balog<sup>1</sup>, M. Wohlgemuth<sup>2</sup>, M. Auranen<sup>3</sup>, S. Mitsuhashi<sup>4</sup>, P. J. van der Vliet<sup>1</sup>, K. Straasheim<sup>1</sup>, R. F. P. van den Akker<sup>1</sup>, M. Kriek<sup>1</sup>, M. E. Y. Laurensse-Bik<sup>1</sup>, V. Raz<sup>1</sup>, M. M. van Ostaaijen - ten Dam<sup>1</sup>, K. B. M. Hansson<sup>1</sup>, E. L. van der Kooi<sup>5</sup>, S. Kiuru-Enari<sup>3</sup>, B. Udd<sup>6</sup>, M. J. D. van Tol<sup>7</sup>, I. Nishino<sup>8</sup>, R. Tawil<sup>7</sup>, S. J. Tapscott<sup>9</sup>, B. G. M. van Engelen<sup>2</sup>, S. M. van der Maarel<sup>1</sup>;*

<sup>1</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>2</sup>Radboud University Medical Centre, Nijmegen, Netherlands, <sup>3</sup>University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>4</sup>National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan, <sup>5</sup>Medisch Centrum Leeuwarden, Leeuwarden, Netherlands, <sup>6</sup>Tampere University Hospital and University of Tampere, Tampere, Finland, <sup>7</sup>University of Rochester Medical Center, Rochester, NY, United States, <sup>8</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

Facioscapulohumeral muscular dystrophy (FSHD) is associated with the partial chromatin relaxation of the *DUX4* encoding D4Z4 macrosatellite repeat located on chromosome 4, and transcriptional derepression of *DUX4* in skeletal muscle. D4Z4 chromatin relaxation is consistently marked by CpG hypomethylation at the *DUX4* promoter.

In the most common form, FSHD1, D4Z4 chromatin relaxation is caused by a D4Z4 repeat array contraction to 1-10 units (normal range 8-100 units). In the rare form of FSHD, FSHD2, D4Z4 chromatin relaxation occurs on all D4Z4 arrays and is most often caused by mutations in the D4Z4 chromatin repressor SMCHD1.

Not all FSHD2 cases can be explained by *SMCHD1* mutations, thus we performed whole exome sequencing in FSHD families with D4Z4 hypomethylation lacking *SMCHD1* mutations. In two families we identified heterozygous mutations in *DNMT3B*, which segregated with D4Z4 hypomethylation and increased FSHD penetrance. Recessive *DNMT3B* mutations were previously shown to cause Immunodeficiency, Centromeric instability and Facial anomalies syndrome type 1 (ICF1). These FSHD patients don't have immunodeficiency, but ICF1 patients might be at risk of expressing *DUX4* and developing FSHD. We propose that the effect of *DNMT3B* mutations on *DUX4* expression and disease presentation, like for *SMCHD1*, depends on several aspects associated with the FSHD locus including D4Z4 repeat size, and the presence of a polymorphic *DUX4* polyadenylation signal. This study suggests that multiple factors are involved in the epigenetic state at D4Z4 and the regulation of *DUX4*.

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### C11.4

#### Homozygous mutations in the sterile alpha motif and leucine zipper containing kinase, ZAK, is associated with congenital myopathy with fiber type disproportion

*M. Tetreault<sup>1,2</sup>, E. Harris<sup>3</sup>, N. Vasil<sup>4</sup>, J. Karamchandani<sup>5</sup>, E. Bareke<sup>1,2</sup>, J. Majewski<sup>1,2</sup>, N. B. Romero<sup>6,7</sup>, R. Barresi<sup>8</sup>, E. Malfatti<sup>6,7</sup>, K. Choquet<sup>1,8</sup>, M. Dicaire<sup>9</sup>, Y. Shao<sup>6</sup>, A. Topf<sup>8</sup>, E. O'Ferrall<sup>5</sup>, V. Straub<sup>3</sup>, H. Lochmüller<sup>3</sup>, J. Laporte<sup>6</sup>, B. Brais<sup>1,8</sup>;*

<sup>1</sup>Department of Human Genetics, McGill University, Montreal, QC, Canada, <sup>2</sup>McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada, <sup>3</sup>John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom,

<sup>4</sup>IGBCM (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France, <sup>5</sup>Department of Pathology, McGill University Health Centre, Montreal Neurological Institute Hospital, Montreal, QC, Canada, <sup>6</sup>Université Sorbonne, UPMC Univ Paris 06,

<sup>7</sup>INSERM UMRS974, CNRS FRE3617, Center for Research in Myology, GH Pitié-Salpêtrière, Paris, France, <sup>8</sup>Centre de référence de Pathologie Neuromusculaire Paris-Est, Institut de Myologie, GHU Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France, <sup>9</sup>Rare Neurological Diseases Group, Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, QC, Canada.

**Background:** Congenital myopathies are a heterogeneous group of diseases characterized by early childhood hypotonia and muscle weakness. They are sub-classified in seven distinct types based on clinical presentation and characteristic features on biopsy. To date, more than 30 genes have been associated with congenital myopathies and a large number of patients are still without a diagnostic.

**Methods:** In order to identify the causative gene, three consanguineous families, from different ethnic backgrounds, were exome sequenced independently. RNA-sequencing was also performed on muscle biopsy from one affected.

**Results:** Whole-exome sequencing identified homozygous truncating mutations in ZAK, encoding for the sterile alpha motif and leucine zipper containing kinase. RNA-sequencing confirmed a decreased expression of ZAK in muscle, suggesting mRNA decay. Transcriptome analysis showed similarities of affected pathways with other muscle diseases including the up-regulation of extracellular matrix components as well as a down-regulation of sarcomeric genes.

**Conclusion:** In this study, we linked a loss-of-function mutation in ZAK with congenital myopathy. Although transcriptome analysis was performed on only one patient, we obtained results suggesting affected pathways common to other myopathies. The identification of mutations in ZAK will allow accurate genetic counseling and potentially lead to the identification of novel therapeutic targets.

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### C11.5

#### Expanding the allelic and locus heterogeneity of tRNA synthetase-related neuromuscular disease

*A. Antonelli<sup>1</sup>, L. Griffin<sup>1</sup>, S. Oprescu<sup>1</sup>, M. Shy<sup>2</sup>, M. Meisler<sup>1</sup>, J. Kitzman<sup>1</sup>, A. Beg<sup>1</sup>;*

<sup>1</sup>University of Michigan, Ann Arbor, MI, United States, <sup>2</sup>University of Iowa, Iowa City, IA, United States.

Four genes encoding aminoacyl-tRNA synthetases (ARSs) cause dominant, axonal Charcot-Marie-Tooth (CMT) disease, which is characterized by impaired motor and sensory function in the distal extremities. ARS genes implicated in dominant CMT disease thus far are glycyl-(GARS), tyrosyl-(YARS), alanyl-(AARS), and histidyl-tRNA synthetase (HARS). Functional analyses revealed that CMT-associated ARS mutations reduce enzymatic activity, suggesting that impaired tRNA charging is a component of disease pathogenesis. Currently, ARS variants of unknown pathogenicity are being discovered rapidly, making it important to proactively determine the subset of variants relevant to CMT disease. We performed a forward mutation screen in yeast that revealed 17 novel loss-of-function GARS and AARS mutations, one of which was subsequently found in a patient with CMT disease. These functional data contributed to a rapid patient diagnosis. We are expanding these efforts via programmed allelic series (PALS) in allelically heterogeneous yeast growth assays. If impaired tRNA charging causes CMT disease, then mutations in other ARS genes may cause this phenotype. To test this, we identified loss-of-function missense mutations in an ARS not yet associated with disease [threonyl-(TARS)] and evaluated them for dominant toxicity in *C. elegans*. This revealed two classes of mutations: those toxic to neurons and those not toxic to neurons. These "separation of function" mutations will be essential for determining the molecular pathology of ARS-associated CMT disease, which we are pursuing in mouse. Combined, our data suggest that mutations in a broader panel of ARS genes cause dominant CMT disease and that improving ARS function is a relevant therapeutic strategy.

### C11.6

#### *De novo* mutations in PDE10A cause childhood-onset chorea with bilateral striatal lesions

*N. E. Mencacci<sup>1,2</sup>, E. Kamsteeg<sup>3</sup>, K. Nakashima<sup>4</sup>, L. R'Bilo<sup>1</sup>, D. S. Lynch<sup>1</sup>, B. Balint<sup>1,5</sup>, M. A. P. Willemens<sup>3</sup>, M. E. Adams<sup>6</sup>, S. Wiethoff<sup>7,8</sup>, K. Suzuki<sup>4</sup>, C. H. Davies<sup>8</sup>, J. Ng<sup>9</sup>, E. Meyer<sup>9</sup>,*

*L. Veneziano<sup>10</sup>, P. Giunti<sup>11</sup>, D. Hughes<sup>1</sup>, F. L. Raymond<sup>11</sup>, M. Carecchio<sup>12</sup>, G. Zorzi<sup>12</sup>, N. Nardocci<sup>12</sup>, C. Barzaghi<sup>12</sup>, B. Garavaglia<sup>12</sup>, V. Salpietro<sup>1</sup>, J. Hardy<sup>1</sup>, A. M. Pittman<sup>1</sup>, H. Houlden<sup>1</sup>, M. A. Kurian<sup>9,13</sup>, H. Kimura<sup>4</sup>, N. W. Wood<sup>1</sup>, K. P. Bhatia<sup>14</sup>, L. E. L. M. Vissers<sup>1</sup>,*

<sup>1</sup>UCL Institute of Neurology, London, United Kingdom, <sup>2</sup>IRCCS Istituto Auxologico Italiano, Universita' degli Studi di Milano, Milan, Italy, <sup>3</sup>Radboudumc, Nijmegen,

Netherlands, <sup>4</sup>Takeda Pharmaceutical Company Limited, Fujisawa, Japan, <sup>5</sup>University Hospital Heidelberg, Heidelberg, Germany, <sup>6</sup>National hospital for neurology and neurosurgery, London, United Kingdom, <sup>7</sup>Eberhard-Karls-University, Tübingen, Germany, <sup>8</sup>4CNS Drug Discovery Unit, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, Fujisawa, Japan, <sup>9</sup>UCL Institute of Child Health, London, United Kingdom, <sup>10</sup>National Research Council, Rome, Italy, <sup>11</sup>University of Cambridge, Cambridge, United Kingdom, <sup>12</sup>IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, <sup>13</sup>Great Ormond Street Hospital, London, United Kingdom, <sup>14</sup>Institute of Neurology, London, United Kingdom.

Chorea is a hyperkinetic movement disorder resulting from dysfunction of striatal medium spiny neurons (MSNs), which form the main basal ganglia output projections. Here we used whole exome sequencing to unravel the underlying genetic cause in three unrelated individuals with a very similar and unique clinical presentation of childhood-onset chorea and characteristic brain MRI showing symmetrical bilateral striatal lesions. All cases were identified to carry a *de novo* heterozygous mutation in *PDE10A* (c.898T>C; p.Phe300Leu in two cases and c.1000T>C; p.Phe334Leu in one case), encoding a phosphodiesterase highly and selectively expressed in MSNs. *PDE10A* contributes to the regulation of the intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Both substitutions affect highly conserved amino acids located in the regulatory GAF-B domain, which, by binding to cAMP, stimulates the activity of the *PDE10A* catalytic domain. *In silico* modeling shows that the mutated residues are located deep into the binding pocket, where they are likely to alter cAMP binding properties. *In vitro* functional studies showed that both substitutions do not affect the basal *PDE10A* activity, but severely disrupt the stimulatory effect mediated by cAMP binding to the GAF-B domain. The identification of *PDE10A* mutations as a cause of chorea further motivates the study of cAMP signaling in MSNs, and highlights the crucial role of striatal cAMP signaling in the regulation of basal ganglia circuitry. Pharmacological modulation of this pathway may offer promising aetiologically-targeted treatments for chorea and other hyperkinetic movement disorders.

## C12 Molecular Dysmorphology

### C12.1

#### Mutations in MAP3K7 and TAB2 that alter the activity of the TAK1 signalling complex cause frontometaphyseal dysplasia

*E. M. Wade*<sup>1</sup>, *P. B. Daniel*<sup>1</sup>, *Z. A. Jenkins*<sup>1</sup>, *A. McInerney-Leo*<sup>2</sup>, *P. Leo*<sup>2</sup>, *T. Morgan*<sup>1</sup>, *M. C. Addor*<sup>3</sup>, *L. C. Adès*<sup>4</sup>, *D. Bertola*<sup>5</sup>, *A. Böhring*<sup>6</sup>, *E. Carter*<sup>7</sup>, *T. Cho*<sup>8</sup>, *R. Conway*<sup>9</sup>, *H. Duba*<sup>10</sup>, *E. Fletcher*<sup>11</sup>, *C. A. Kim*<sup>12</sup>, *D. Krakow*<sup>12</sup>, *T. Neuhann*<sup>13</sup>, *A. Superti-Furga*<sup>14</sup>, *I. Veenstra-Knol*<sup>15</sup>, *D. Wieczorek*<sup>16</sup>, *L. C. Wilson*<sup>17</sup>, *R. C. M. Hennekam*<sup>18</sup>, *A. J. Sutherland-Smith*<sup>19</sup>, *T. M. Strom*<sup>20</sup>, *A. O. M. Wilkie*<sup>21</sup>, *M. A. Brown*<sup>2</sup>, *E. L. Duncan*<sup>2,22</sup>, *D. M. Markie*<sup>1</sup>, *S. P. Robertson*<sup>1</sup>, <sup>1</sup>University of Otago, Dunedin, New Zealand, <sup>2</sup>University of Queensland, Woolloongabba, Australia, <sup>3</sup>Service de Génétique Médicale Maternité, Lausanne, Switzerland, <sup>4</sup>University of Sydney, Sydney, Australia, <sup>5</sup>Universidade de São Paulo, São Paulo, Brazil, <sup>6</sup>Universitätsklinikum Münster, Münster, Germany, <sup>7</sup>Hospital for Special Surgery, New York, NY, United States, <sup>8</sup>Seoul National University Children's Hospital, Seoul, Korea, Republic of, <sup>9</sup>Detroit Medical Center, Detroit, MI, United States, <sup>10</sup>Kepler Universitätsklinikum, Linz, Austria, <sup>11</sup>Western General Hospital, Edinburgh, United Kingdom, <sup>12</sup>UCLA Medical Centre, Santa Monica, CA, United States, <sup>13</sup>Medical Genetics Center, Munich, Germany, <sup>14</sup>University of Lausanne, Lausanne, Switzerland, <sup>15</sup>University Medical Center, Groningen, Netherlands, <sup>16</sup>Heinrich Heine University, Düsseldorf, Germany, <sup>17</sup>Great Ormond Street Hospital, London, United Kingdom, <sup>18</sup>University of Amsterdam, Amsterdam, Netherlands, <sup>19</sup>Massey University, Palmerston North, New Zealand, <sup>20</sup>Helmholtz Zentrum München, Munich, Germany, <sup>21</sup>John Radcliffe Hospital, Oxford, United Kingdom, <sup>22</sup>Royal Brisbane and Women's Hospital, Brisbane, Australia.

Frontometaphyseal dysplasia (FMD) is a sclerosing skeletal dysplasia affecting the long bones and the skull, however individuals present with a range of developmental abnormalities. Approximately 50% of instances of FMD are allelic to the otopalatodigital spectrum disorders caused by gain-of-function mutations in *FLNA*, the mechanism by which these mutations result in hyperostosis is unknown. Using whole-exome and targeted Sanger sequencing in 20 cases of *FLNA*-negative FMD, we found three mutations in genes encoding two components of the TGFβ-activated-kinase (TAK1) complex. Fifteen individuals had the same mutation, c.1454C>T, in *MAP3K7* which encodes TAK1 kinase. A second *de novo* mutation in *MAP3K7*, c.502G>C was discovered in a single individual. A second gene, *TAB2* which encodes TAK1-binding-protein-2, was shown to have the *de novo* mutation, c.1705G>A in a single instance. TAK1, a MAP-3-kinase, is activated via autophosphorylation within its kinase domain. We show the TAK1 mutations are gain-of-function, resulting in a hyper-phosphorylated protein when expressed with TAB2. All three mutations variably alter the activity of more than one signalling pathway regulated by this kinase complex. This includes increased p38 MAPK activation, a key pathway which regulates osteoblastogenesis. These findings

show dysregulation of the TAK1 signalling complex produces a close phenocopy of FMD caused by mutations in *FLNA*. Furthermore they suggest that the pathogenesis of some of the filaminopathies caused by *FLNA* mutations may be mediated by misregulation of signalling co-ordinated through the TAK1 signalling complex.

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### C12.2

#### Mutations in *MYT1*, encoding the myelin transcription factor 1, are a rare cause of Goldenhar syndrome within the RA signalling pathway

*M. Berenger*<sup>1</sup>, *E. Lopez*<sup>1</sup>, *A. Tingaud-Sequeira*<sup>1</sup>, *S. Marlin*<sup>2</sup>, *A. Toutain*<sup>3</sup>, *F. Denoyelle*<sup>4</sup>, *A. Picard*<sup>5</sup>, *S. Charron*<sup>1</sup>, *G. Mathieu*<sup>1</sup>, *H. de Belvalet*<sup>1</sup>, *B. Arveiler*<sup>4,6</sup>, *P. J. Babin*<sup>1</sup>, *D. Lacombe*<sup>1,6</sup>, *C. Rooryck*<sup>1,6</sup>

<sup>1</sup>Univ. Bordeaux, Maladies Rares: Génétique et Métabolisme (MRGM), U 1211 INSERM, Bordeaux, France, <sup>2</sup>Hôpital Universitaire Necker-Enfants-Malades, Département de Génétique, Centre de Référence des Surdités Génétiques, Paris, France, <sup>3</sup>Hôpital Bretonneau, Service de Génétique, Centre Hospitalier Universitaire, Tours, France, <sup>4</sup>Hôpital Universitaire Necker-Enfants-Malades, Service d'ORL pédiatrique et de chirurgie cervicofaciale, Centre de Référence des malformations ORL rares, Paris, France, <sup>5</sup>Hôpital Universitaire Necker-Enfants Malades, Service de chirurgie maxillo-faciale, Paris, France, <sup>6</sup>CHU de Bordeaux, Service de Génétique Médicale, Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Bordeaux, France.

Goldenhar syndrome (GS) or Oculo-Auriculo-Vertebral Spectrum (OAVS) is a developmental disorder involving first and second branchial arches, characterized by asymmetric ear anomalies, hemifacial microsomia, ocular defects, and vertebral malformations. Although numerous chromosomal abnormalities have been associated with OAVS, no causative gene has been identified so far. Among other environmental factors, Retinoic Acid (RA) has already been described as a teratogenic agent leading to OAVS features in humans. As sporadic cases are mostly described in GS, we have performed whole exome sequencing on selected affected individuals and their unaffected parents, looking for *de novo* mutations. Consequently, we identified a heterozygous nonsense mutation in one patient in the *MYT1* gene. Further, we detected one heterozygous missense mutation in another patient from a cohort of 169 OAVS patients. This gene encodes the Myelin Transcription factor 1 which is highly expressed in the developing central nervous system. Functional studies by transient knockdown of *myt1a*, homolog of *MYT1* in zebrafish, led to specific craniofacial cartilages alterations and to the up-regulation of Neural Crest Cells marker *sox10*. Moreover, cells studies confirmed close links between *MYT1*, RA and the RA receptor beta (RAR $\beta$ ). Indeed, All-trans RA (ATRA) treatment led to an upregulation of cellular endogenous *MYT1* expression. Additionally, cellular wild-type *MYT1* overexpression induced a down-regulation of RARB leading to a negative feedback of the RA signalling pathway, whereas mutated *MYT1* did not, confirming the pathogenic effect of the mutations.

Overall, we report *MYT1* as a candidate gene implicated in Goldenhar syndrome, within the RA signalling pathway.

### C12.3

#### A novel gene responsible for Treacher Collins-Franceschetti syndrome

*F. Tran Mau Them*<sup>1</sup>, *B. Laplace-Buïhle*<sup>2</sup>, *C. Collet*<sup>3</sup>, *A. Goldenberg*<sup>4</sup>, *M. Ricquebourg*<sup>2</sup>, *M. Girard*<sup>5</sup>, *E. Sanchez*<sup>1</sup>, *M. Vincent*<sup>6</sup>, *T. Guignard*<sup>5</sup>, *C. Blanchet*<sup>7</sup>, *A. Boland-Auge*<sup>8</sup>, *M. Bihoreau*<sup>9</sup>, *J. Deleuze*<sup>9</sup>, *J. Rivière*<sup>9</sup>, *J. Laplanche*<sup>3</sup>, *G. Captier*<sup>10</sup>, *F. Djouad*<sup>2</sup>, *D. Geneviève*<sup>11</sup>

<sup>1</sup>Département de Génétique Médicale et Inserm U1183, CHRU Montpellier et Institut de Recherche en Génétique et Biologie Moléculaire (IRGBM), Montpellier, France, <sup>2</sup>Unité Inserm U1183, Institut de Recherche en Génétique et Biothérapies (IRGBM), Montpellier, France, <sup>3</sup>UF de Génétique Moléculaire, Service de Biochimie et Biologie Moléculaire, AP-HP Hôpital Lariboisière, Paris, France, <sup>4</sup>Service de Génétique Médicale, CHU Rouen, Rouen, France, <sup>5</sup>Département de Génétique Médicale, CHRU Arnaud de Villeneuve, Montpellier, France, <sup>6</sup>Service de Génétique Médicale, CHU de Nantes, Nantes, France, <sup>7</sup>Service d'ORL, CHRU St Eloi, Montpellier, France, <sup>8</sup>Centre National de Génotypage, Institut de Génétique, CEA, Evry, France, <sup>9</sup>Équipe GAD - Laboratoire de Génétique Moléculaire, Université de Bourgogne - Plateau technique de Biologie - CHU Dijon, Dijon, France, <sup>10</sup>Service de Chirurgie Plastique Infantile, CHRU Lapeyronie, Montpellier, France.

Treacher Collins/Franceschetti syndrome (TCFS; MIM 154500) is a rare genetic disorder. The TCFS is the most common cause of mandibulo-facial dysostosis in human with an estimated prevalence of 1 in 10,000 to 50,000 births. The TCFS is characterized by craniofacial features with bilateral involvement of the first arc brachial responsible for microtia associated with hearing loss, malar and mandibular hypoplasia, cleft palate and other facial abnormalities. To date three genes have been identified as responsible for TCFS, namely TCOF1, POLR1C and POLR1D. These genes are involved in the synthesis of ribosomal RNA and are also important for the differentiation and migration of neural crest cells which lead to the formation of the craniofacial massif. After working for genotyping a large French cohort of TCFS

patients, we realized trio exome in four patients suffering from TCFS and negatives for the three known genes. We identified two different *de novo* non-sense mutations in the same gene. We achieved a morpholino against the RNA of this gene in zebrafish models with a reporter of derived neural crest cells (FOXD3) and cartilage structures (COL2A1). We observed cranio-facial malformations mimicking the human phenotype namely a significant decrease of the mandible and gills and the impression of a decrease in the migration of cells from the neural crest. In conclusion we have identified a 4th gene in STCF inherited in an autosomal dominant manner. This discovery extends the number of ribosomopathies.

#### C12.4

#### Loss of a non-coding regulatory element on chromosome 9 in a family with a Cornelia de Lange syndrome (CdLS)-like phenotype

J. Pozojevic<sup>1</sup>, E. Watrin<sup>2,3</sup>, B. Geidner<sup>4</sup>, I. Parenti<sup>1</sup>, K. S. Wendt<sup>4</sup>, R. Siebert<sup>5</sup>, A. Rakovic<sup>6</sup>, G. Gillessen-Kaesbach<sup>7</sup>, F. J. Kaiser<sup>1</sup>;

<sup>1</sup>Sektion für Funktionelle Genetik am Institut für Humangenetik Lübeck, Universität zu Lübeck, Lübeck, Germany, <sup>2</sup>Centre National de la Recherche Scientifique, UMR 6290, Rennes, France, <sup>3</sup>Institut de Génétique et Développement de Rennes, Université de Rennes 1, SFR BIOSIT, Rennes, France, <sup>4</sup>Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands, <sup>5</sup>Institut für Humangenetik, Christian-Albrechts-Universität Kiel and Universitätsklinikum Schleswig-Holstein, Kiel, Germany, <sup>6</sup>Institute of Neurogenetics, University of Lübeck, Lübeck, Germany, <sup>7</sup>Institut für Humangenetik, Universität zu Lübeck, Lübeck, Germany.

CdLS is a dominantly inherited malformation syndrome caused by mutations in genes encoding subunits (SMC1A, SMC3, RAD21) or regulators (NIPBL, HDAC8) of the cohesin complex. This DNA-bound complex regulates chromatin-related processes like chromosome segregation, DNA-damage repair, transcription and chromatin structure. Here we report on a family with two children and a mother with clinical features reminiscent of CdLS. While gene panel sequencing approaches failed to identify the disease-causing mutation, an 87 kb spanning deletion co-segregating with the phenotype was identified by array-CGH. Besides the coding region of CYLC2, encoding a sperm head protein, no other genes were affected. Subsequent in-silico analyses predicted the existence of a ~3 kb tissue-specific regulatory element within this region, approximately 1 Mb distant from the next protein-coding gene SMC2, which encodes a subunit of the cohesin-related condensin complex.

Significant reduction of SMC2 expression was verified in patient's fibroblasts by qPCR analysis. Accordingly, a strong dysregulation of SMC2 was observed in SH-SY5Y cells deficient for the putative 3 kb regulatory element, that was deleted by CRISPR/Cas9 genome editing. Reporter gene assays further highlighted the functional relevance of the identified regulatory element in regulating the SMC2 gene promoter. Interestingly, dysregulation of SMC2 correlated with the dysregulation of another condensin subunit SMC4 in patient's samples as well as CRISPR/Cas9-generated cells. Furthermore, siRNA-mediated reduction of SMC2 in different cell types also resulted in a decreased SMC4 expression.

Our data indicate for the first time a putative relevance of condensin for human disease, which might be dosage-sensitive and/or tissue specific.

#### C12.5

#### PCGF2 syndrome—distinctive facies, hypotonia, poor feeding, constipation, thin hair, and global developmental delay with deep cerebral white matter changes—due to *de novo* missense mutations affecting the same conserved proline residue in PCGF2

P. D. Turnpenny<sup>1</sup>, M. Wright<sup>2</sup>, C. Tysoe<sup>1</sup>, T. van Essen<sup>3</sup>, R. Pfundt<sup>4</sup>, S. White<sup>5</sup>, N. Lotan<sup>6</sup>, L. Carpenter<sup>7</sup>, B. Schaefer<sup>8</sup>, W. K. Chung<sup>9</sup>, H. McLaughlin<sup>10</sup>, L. B. Henderson<sup>10</sup>, A. Roberts<sup>11</sup>, R. Gjergja-Juraszki<sup>12</sup>, K. Forbes<sup>13</sup>, A. E. Fry<sup>14</sup>, D. T. Pilz<sup>13</sup>;

<sup>1</sup>Royal Devon & Exeter Hospital, Exeter, United Kingdom, <sup>2</sup>Norther Regional Clinical Genetics, Newcastle, United Kingdom, <sup>3</sup>University of Groningen, Groningen, Netherlands, <sup>4</sup>Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, <sup>5</sup>Victorian Clinical Genetics Services, Melbourne, Australia, <sup>6</sup>Hadassah-Hebrew University Medical Center, Jerusalem, Israel, <sup>7</sup>St Francis Genetics, Tulsa, OK, United States, <sup>8</sup>Arkansas Children's Hospital, Little Rock, AR, United States, <sup>9</sup>Columbia University, New York, NY, United States, <sup>10</sup>GeneDX, Gaithersburg, MD, United States, <sup>11</sup>Boston Children's Hospital, Boston, MA, United States, <sup>12</sup>Children's Hospital Srebrnjak, Zagreb, Croatia, <sup>13</sup>Queen Elizabeth University Hospital, Glasgow, United Kingdom, <sup>14</sup>University Hospital of Wales, Cardiff, United Kingdom.

We report 10 subjects, 4 males and 6 females aged 3-18 years with 'PCGF2 syndrome', ascertained worldwide following the identification of 2 cases through the DDD Study by whole exome sequencing of family trios. All individuals have a *de novo* missense mutation in a novel gene, PCGF2, affecting the same highly conserved proline residue; eight with p.P65L and MZ twins with p.P65S. The range of features and intellectual disability, including MRI brain imaging, is now evident. Most have a highly distinctive flat and myo-

pathic facial profile with malar hypoplasia, short palpebral fissures, unusual ear helices, and small oral stoma. Birth weight was <10<sup>th</sup> centile in 8/10; height <10<sup>th</sup> in 4/10; and weight <10<sup>th</sup> in 8/10. Head circumference varied widely: <10<sup>th</sup> centile in 4/10 and ≥90<sup>th</sup> in 3/10. Some had minor skeletal anomalies, including three with kyphosis/scoliosis and/or formation anomalies of the spine. All have global developmental delay, speech delay (two non-verbal), hypotonia, poor feeding, constipation, and thin hair to a variable degree. Neuroimaging, available in 7/10 patients, showed extensive areas of abnormal white matter; in the MZ twins and 1 other patient this was associated with polymicrogyria.

PCGF2 encodes a polycomb group ring finger protein. Polycomb group proteins form multimeric complexes and repress target gene expression. PCGF2 has a known role as a tumour suppressor; however, this is the first report of a consistent, syndromic neurodevelopmental phenotype in humans with neuroimaging anomalies caused by germline mutations in the gene.

#### C12.6

#### Genetic and functional comparison of identical germline and somatic SETBP1 mutations

R. Acuna-Hidalgo<sup>1</sup>, P. Deriziotis<sup>2</sup>, G. M. Findlay<sup>3</sup>, C. Gilissen<sup>1</sup>, International Schinzel-Giedion syndrome collaboration, J. A. Veltman<sup>1,4</sup>, S. E. Fisher<sup>2</sup>, J. Shendre<sup>3</sup>, A. Hoischen<sup>1</sup>, B. W. van Bon<sup>1</sup>;

<sup>1</sup>Department of Human Genetics, Radboud UMC, Nijmegen, Netherlands, <sup>2</sup>Department of Language and Genetics, Max Planck Institute for Psycholinguistics, Nijmegen, Netherlands, <sup>3</sup>Department of Genome Sciences, University of Washington, Seattle, WA, United States, <sup>4</sup>Department of Clinical Genetics, University of Maastricht, Maastricht, Netherlands.

Schinzel-Giedion syndrome (SGS) is a rare severe developmental disorder characterized by malformations in multiple organ systems. SGS is caused by *de novo* germline mutations clustering within a 12-bp exonic region in SETBP1. Interestingly, identical mutations in SETBP1 occur somatically in myeloid leukemia, leading to increased cell proliferation. The aim of our study is to analyze comparatively germline and somatic SETBP1 mutations to gain insight into genotype-phenotype correlations for SGS and into the molecular basis of SGS.

We have gathered clinical information from over 45 SGS patients, constituting the largest SGS cohort yet. Comparing SETBP1 mutations in SGS and in leukemia shows that, despite sharing an overlap, the distribution of mutations within the hotspot differs between both conditions. Overexpression experiments confirm that mutations in the SETBP1 hotspot destroy a degron and lead to increased SETBP1 protein, with different mutations having varying effects on SETBP1 stability and protein levels. Furthermore, we perform saturation genome editing (Findlay et al. Nature 2014) to analyze the effect of all theoretically possible mutations in the SETBP1 hotspot and surrounding region. In silico modeling and functional studies substantiate that mutations seen more frequently in SGS are functionally weaker than those more prevalent in leukemia. Clinical data supports this finding; SGS patients with germline SETBP1 mutations shown to be functionally stronger and more prevalent in leukemia show increased cell proliferation in vitro, increased incidence of malignancy and decreased survival.

Our results support a correlation between the genotype, molecular and cellular consequences and the phenotype in germline SETBP1 mutations.

### C13 Prenatal Genetics and carrier screening

#### C13.1

#### Validation of non-invasive prenatal diagnosis (NIPD) of multiple single gene disorders for clinical implementation

M. Parks, S. Court, S. Cleary, S. Clokie, J. Hewitt, D. Williams, T. Cole, F. MacDonald, M. Griffiths, S. Allen; Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom.

In an effort to implement NIPD for single gene disorders (SGDs) into clinical practice (NIPSIGEN project), we have recently developed and validated an NIPD test for Duchenne and Becker muscular dystrophies (DMD/BMD). Building on this experience, we have now designed an improved method for the NIPD of multiple SGDs, namely DMD/BMD, spinal muscular atrophy (SMA) and congenital adrenal hyperplasia (CAH). This was achieved through targeted enrichment of 9054 highly heterozygous SNPs in total (800 Kb of captured genomic region across 6 Mb windows on ChrX, Chr5, and Chr6) and massively parallel sequencing of cfDNA followed by relative haplotype dosage (RHDO) analysis. Maternal, paternal and proband genomic DNA samples were also tested alongside cfDNA for haplotype phasing and to measure the fetal fraction. The test achieved 100% sensitivity and speci-

ficity on patients tested so far, which included four pregnancies at risk of DMD/BMD, five at risk of SMA, and nine healthy control pregnancies (where the fetal DNA obtained from the CVS was used to simulate the proband DNA in seven cases for DMD/BMD testing, and in two cases for SMA and CAH testing). Our improved method is capable of testing 2-3 patients on a single MiSeq run for any of these three SGDs, thus increasing the multiplexing capacity and decreasing testing costs for clinical laboratories. This leads us to believe that NIPD for the most common SGDs might soon become available as a clinical service to patients in the UK and elsewhere.

Funded by: Health Innovation Challenge Fund (DoH, Wellcome Trust).

### C13.2

#### Non-invasive prenatal testing of single-gene disorders: fact or fiction?

Y. Rozenholc<sup>1</sup>, L. Orhant<sup>2</sup>, F. Leturcq<sup>2,3</sup>, E. Girodon<sup>2</sup>, C. Costa<sup>2</sup>, D. Vidaud<sup>2,4</sup>, M. Vidaud<sup>2,4</sup>, D. Desaule<sup>1</sup>, T. BienvENU<sup>2,5</sup>, J. Nectoux<sup>2,5</sup>,

<sup>1</sup>MERIT - UMR IRD 216, Sorbonne Paris Cité, Université Paris Descartes, Faculté de Pharmacie de Paris, Paris, France, <sup>2</sup>Biochimie et Génétique Moléculaire, HUPC Hôpital Cochin, Paris, France, <sup>3</sup>Sorbonne Universités, UPMC Université Paris 06, INSERM UMR974, CNRS FRE3617, Centre de recherche en Myologie, Paris, France, <sup>4</sup>EA7331, Faculté de Pharmacie Paris, Université Paris Descartes Sorbonne Paris Cité, Paris, France, <sup>5</sup>INSERM, U1016, Institut Cochin, CNRS UMR8104, Université Paris Descartes, Paris, France.

**Background.** In recent years, the deployment of technologies for the analysis of cell-free fetal DNA, such as digital-PCR and NGS, allowed for the implementation of various non-invasive prenatal tests in clinical practice and opened the way to the diagnosis of single-gene disorders. However, to date, it has only been offered for paternal and *de novo* mutation exclusion, the study of the maternal inheritance remaining challenging because of the high levels of maternal background.

**Methods.** We collected 190 plasma samples from women that underwent a classic invasive prenatal diagnosis for Achondroplasia, Cystic-Fibrosis, Duchenne Myopathy, Hemophilia or Neurofibromatosis Type 1. Digital-droplet PCR assays specifically designed for wild-type and mutant alleles were used to evaluate the observed allelic balance in maternal plasma. A precisely derived Likelihood Ratio Test, taking account of the inheritance pattern, the nature of the mutation and the observed fetal fraction, was used to infer the fetal genotype.

**Results.** Concerning *de novo* and paternally-inherited mutations, fetal genotype was determined correctly in 100% of the tested cases (49 samples) with fetal fraction >1%. About maternally-inherited mutations, we showed that the percentage of conclusive results highly depends both on the available amount of cell-free DNA and the fetal fraction, thus leading us to propose recommendations in terms of blood volume to collect, for each type of single-gene disorder.

**Conclusion.** This proof-of-concept study of non-invasive droplet-digital PCR technology for the analysis of both paternally and maternally inherited fetal alleles demonstrates that NIPT for single-gene disorders is now becoming achievable.

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### C13.3

#### Maternal incidental findings during non-invasive prenatal testing for fetal aneuploidies

N. Brison<sup>1</sup>, K. Van Den Bogaert<sup>1</sup>, J. M. E. Van den Oever<sup>2</sup>, L. Dehaspe<sup>1</sup>, K. Janssens<sup>3</sup>, B. Blaumeiser<sup>3</sup>, H. Peeters<sup>1</sup>, H. Van Esch<sup>1</sup>, G. Van Buggenhout<sup>1</sup>, A. Vogels<sup>1</sup>, T. de Ravel<sup>1</sup>, E. Legius<sup>1</sup>, K. Devriendt<sup>1</sup>, J. R. Vermeesch<sup>1</sup>;

<sup>1</sup>Centre for Human Genetics, UZ Leuven, Leuven, Belgium, <sup>2</sup>Leiden University Medical Center, dept. of Clinical Genetics, Leiden, Netherlands, <sup>3</sup>Centre for Medical Genetics, University and University Hospital Antwerp, Antwerp, Belgium.

Non-invasive prenatal testing (NIPT) for common fetal aneuploidies by either targeted or whole genome sequencing of circulating free DNA (cfDNA) has become standard prenatal care. Random genome-wide cfDNA sequencing enables not only the detection of fetal chromosomal imbalances but also of maternally derived copy number variants (CNVs). Following routine clinical analysis of over 10,000 prospective pregnancies using an accredited in-house developed analysis pipeline we identified five different clinically relevant constitutional CNVs and imbalanced translocations of maternal origin which were reported back to the mother because they (i) were either relevant for the pregnancy management, (ii) had potential consequences for the fetus or (iii) could have consequences for future reproductive choice. Hence, genome wide cfDNA profiling of maternal plasma improves not only fetal but also more general pregnancy management.

### C13.4

#### A prenatal targeted exome sequencing approach - Fetalis - designed specifically for fetuses with ultrasound abnormalities reveals an important fraction of cases with associated gene defects

C. Pangalos<sup>1</sup>, B. Hagnefelt<sup>1</sup>, K. Lilakos<sup>2</sup>, C. Konialis<sup>1</sup>;

<sup>1</sup>InterGenetics-Diagnostic Genetics Center, Athens, Greece, <sup>2</sup>Department of Haematology, University of Athens Medical School, Athens, Greece.

**Introduction:** The underlying genetic cause of fetal malformations and other fetal structural abnormalities is often elusive, leading to an inability to provide a precise diagnosis and accurate reproductive and fetal risk assessment. We report the development and first clinical applications of an expanded exome sequencing-based test, coupled to a bioinformatics-driven prioritization algorithm, targeting gene disorders presenting with abnormal prenatal ultrasound findings.

**Materials and Methods:** We applied the testing strategy to 14 euploid fetuses, from 11 on-going pregnancies and 3 products of abortion, all with various ultrasound abnormalities. Whole exome sequencing (WES) was followed by variant prioritization, utilizing a custom analysis pipeline (Fetalis algorithm), targeting 758 genes associated with genetic disorders which may present with abnormal fetal ultrasound findings.

**Results:** A definitive or highly-likely diagnosis was made in 6 of 14 cases (43%), involving 3 abortuses and 3 on-going pregnancies. In the remaining 8 on-going pregnancy cases (57%), a ZIC1 variant of unknown clinical significance was detected in one case, while 7 cases did not harbor pathogenic variant(s). Pregnancies were followed-up to birth, resulting in one neonate harboring the PROKR2 mutation and presenting with isolated minor structural cardiac abnormalities, and in 7 apparently healthy neonates.

**Conclusions:** The expanded targeted exome sequencing-based approach described herein (Fetalis), provides strong evidence suggesting a definite and beneficial increase in our diagnostic capabilities in prenatal diagnosis of chromosomally balanced fetuses with troubling ultrasound abnormalities. Furthermore, the proposed strategy, overcomes many of the problems and limitations associated with clinical wide-scale WES testing in a prenatal setting.

### C13.5

#### Exome sequencing of parental samples is a powerful strategy for the diagnosis of lethal recessive disorders

K. L. Stals<sup>1</sup>, J. Baptista<sup>1</sup>, A. Bussell<sup>1</sup>, R. Caswell<sup>2</sup>, M. Johnson<sup>2</sup>, G. Jones<sup>1</sup>, H. Lango Allen<sup>2</sup>, A. Parrish<sup>1</sup>, C. Tysoe<sup>1</sup>, M. Wakeling<sup>2</sup>, S. Ellard<sup>1,2</sup>;

<sup>1</sup>Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom, <sup>2</sup>Institute of Biomedical and Clinical Science, University of Exeter, Exeter, United Kingdom.

Rare disorders resulting in prenatal or neonatal death are both phenotypically and genetically heterogeneous, making these disorders difficult to diagnose. Some affected fetuses can be diagnosed by ultrasound scan but often not until mid-gestation of a pregnancy and there is generally only a limited quality and quantity of fetal DNA available for genetic testing. We previously reported a novel strategy of sequencing parental DNA samples to identify potential causative heterozygous mutations that were confirmed as biallelic in the affected fetuses of two couples. We now report exome analysis in a further 19 couples (total cohort 21 couples) who had previous pregnancies affected with a lethal disorder. Likely or definitely causative mutations were identified in 14/21 couples (67%) and where two or more fetuses were affected a genetic diagnosis was obtained in 11/14 (78%) cases. Mutations were identified in 12 different genes (*RYR1* n=2, *GLE1* n=2, *MRPS22*, *CENP*, *LRP4*, *BBS9*, *BBS10*, *SASS6*, *DYNC2H1*, *B3GALT1*, *FRAS1* and *ITGA8*). In most cases the reported phenotypes associated with these genes are consistently lethal, but the mutations identified in the *RYR1* and *LRP4* genes represent the most severe form of a variable phenotypic spectrum. Eight referrals involved an ongoing pregnancy, with five diagnoses reported in time to inform management. At least one couple has had prenatal testing in a subsequent pregnancy. Exome sequencing of parental samples is a highly effective strategy for the diagnosis of lethal recessive disorders in outbred couples where there is limited fetal DNA available for testing.

## C13.6

**Application of a NGS assay for the identification of individuals carrying recessive genetic mutations in reproductive medicine**

*B. Rodríguez-Santiago<sup>1</sup>, A. Abulí<sup>2</sup>, M. Boada<sup>3</sup>, M. García<sup>1</sup>, S. Carbonell<sup>1</sup>, B. Coroleu<sup>3</sup>, A. Veiga<sup>3</sup>, P. N. Barrí<sup>3</sup>, X. Estivill<sup>2,4,5</sup>, L. A. Pérez-Jurado<sup>4,6,7</sup>, L. Armengol<sup>1</sup>;*

<sup>1</sup>*qGenomics, Espugues del Llobregat, Spain, <sup>2</sup>Unit of Medical Genomics, Department of Obstetrics, Gynaecology and Reproduction, Dexeus Women's Health, Barcelona, Spain, <sup>3</sup>Service of Reproduction Medicine, Obstetrics, Department of Obstetrics, Gynaecology and Reproduction, Dexeus Women's Health, Barcelona, Spain, <sup>4</sup>Genetics Unit, Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), Barcelona, Spain, <sup>5</sup>Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, <sup>6</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>7</sup>CIBER in Rare Disorders (CIBERER), Barcelona, Spain.*

**Introduction:** One of the main challenges of current genetic medicine is to provide preventive actions. Utilisation of next-generation sequencing technologies (NGS) for screening carriers of recessive mutations has the capacity of providing information to make medical interventions in gamete donation programs.

**Methods:** We have developed and validated a NGS carrier-screening test (qCarrier test) that includes 200 genes associated with 218 autosomal recessive and 22 X-linked diseases. Carrier screening is performed to oocyte donation candidates and the male partner of oocyte recipient. Carriers of X-linked conditions are discarded from the program, while donors are chosen not carrying mutations for the same gene/disease as the recipients.

**Results:** The validation phase showed a high sensitivity (>99%) detecting all single nucleotide variants, 13 indels and 25 copy number variants included in the validation set. A total of 1,301 individuals were analysed with the qCarrier test, including 483 candidate oocyte donors and 635 receptor couples, 105 females receiving sperm donation and 39 couples seeking pregnancy. We identified 56% of individuals who are carriers for at least one genetic condition and 1,7% of female donors who were discarded from the program due to a carrier state of X-linked conditions. Globally, 3% of a priori assigned donations had a high reproductive risk that could be minimized after testing. Genetic counselling at different stages is essential in helping to a successful and healthy pregnancy.

## C14 Cancer Genetics: From modeling to profiling

## C14.1

**LYNCH SYNDROME MOUSE: A MODEL FOR COLON CARCINOGENESIS**

*M. Pussila<sup>1</sup>, P. Törönen<sup>1</sup>, E. Einarsdóttir<sup>2,3</sup>, S. Katayama<sup>2</sup>, J. Linden<sup>4</sup>, J. Kere<sup>2,3</sup>, M. Nyström<sup>1</sup>;*

<sup>1</sup>*Department of Biosciences, University of Helsinki, Helsinki, Finland, <sup>2</sup>Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden, <sup>3</sup>Folkhälsan Institute of Genetics, University of Helsinki, Helsinki, Finland, <sup>4</sup>Department of Basic Veterinary Sciences, University of Helsinki, Helsinki, Finland.*

Western-style diet (WD), which is rich in fat and scarce of fiber and vitamin D, predispose to colorectal cancer (CRC). We performed a long-term diet experiment with WD in the mouse to address putative cancer-predisposing events in colonic mucosa. Transcriptome-wide gene expression profiles were studied for 12 and 18 month old mice analogous to human Lynch syndrome (LS) (Mlh1<sup>+/−</sup>), and their wild type littermates (Mlh1<sup>+/+</sup>). Furthermore, tumor phenotypes, microsatellite instability (MSI) and mismatch repair protein (MMR) loss, linked to LS were characterized. Altogether 6 CRCs were detected and 5 of them in WD fed mice. Interestingly, our results show that expression profiles of histologically normal mucosa from mice which developed CRC form a distinct cluster compared to healthy controls in MDS plot created with 100 most regulated genes in the CRC developing samples. The genes were selected with shrinkage-T test. This kind of clustering clearly indicates a colon-wide field effect of CRC-predisposing events in the normal colon mucosa. It is assumed that MMR gene inactivation and consequent tumor initiation follows the classical two-hit model. Our results, however, suggest that already decreased amount of Mlh1, when MMR is still intact, may be sufficient to initiate tumorigenesis. Lynch syndrome mice did not lack Mlh1 or show MSI in their tumors, although Mlh1 RNA expression was already significantly decreased in their mucosa.

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## C14.2

**Frameshift mutations in microsatellite unstable colorectal cancers: from immune signature to personalized immunotherapy**

*P. Maby<sup>1,2</sup>, H. Kora<sup>1</sup>, M. Hamieh<sup>1,3</sup>, D. Tougeron<sup>1,4</sup>, G. Bindea<sup>2</sup>, B. Mlecník<sup>2</sup>, H. Angell<sup>2,5</sup>, T. Fredriksen<sup>2</sup>, N. Elie<sup>6</sup>, J. Leprince<sup>7</sup>, J. Mauillon<sup>8,9</sup>, F. Le Pessot<sup>10</sup>, R. Sesboué<sup>6</sup>, T. Frebourg<sup>1,8</sup>, J. Galon<sup>2</sup>, J. Latouche<sup>1,8</sup>;*

<sup>1</sup>*Inserm U1079-IRIB, Normandy Centre for Medical Genomics and Personalized Medicine, Rouen University, Rouen, France, <sup>2</sup>Inserm U1138, Paris Descartes University, Pierre et Marie Curie University, Paris, France, <sup>3</sup>The Center for Cell Engineering, Memorial Sloan Kettering Cancer Center, New York, NY, United States, <sup>4</sup>Department of Gastroenterology, Poitiers University Hospital and EA 4331, Poitiers University, Poitiers, France, <sup>5</sup>AstraZeneca Pharmaceuticals, Cambridge, United Kingdom, <sup>6</sup>Imaging Core Facility, Caen University Hospital, Caen, France, <sup>7</sup>Inserm U982-IRIB, Cell Imaging Platform of Normandy PRIMACEN, Rouen University, Mont-Saint-Aignan, France, <sup>8</sup>Department of Genetics, Rouen University Hospital, Rouen, France, <sup>9</sup>Department of Gastroenterology, Le Havre Hospital, Le Havre, France, <sup>10</sup>Department of Pathology, Rouen University Hospital, Rouen, France.*

Microsatellite instability (MSI), due to DNA mismatch repair (MMR) machinery deficiency, is found in 15% of all colorectal cancers (CRCs), including Lynch syndrome, the most frequent hereditary form of CRC. In MSI-CRCs, overall tumor-infiltrating lymphocyte (TIL) density and survival rate are known to be higher than in microsatellite stable CRCs (MSS-CRCs). Indeed, frameshift mutations within coding repeat sequences could lead to the synthesis of immunogenic neo-antigens recognized by anti-tumor CD8+ T lymphocytes. However, the clear links between MMR machinery deficiency, TIL density and prognosis remain to be established.

Starting from 141 MSI-CRCs, we showed by gene expression profiling and immunohistochemistry, that these cancers, compared to MSS-CRCs, expressed more immune-related genes and were infiltrated with more *in situ* proliferative T cells, functional CD8+ T cells, B cells and macrophages, which correlated with prolonged survival. Moreover, we found that CD8+ TIL density was associated with the total number of tumor frameshift mutations (FSMs), characterized by multiplex PCRs, and was especially higher when a FSM was present in *ASTE1*, *HNF1A* or *TCF7L2* gene. Finally, using artificial antigen presenting cells developed in the laboratory, starting from peripheral blood of HLA-A2+ MSI-CRC Lynch patients, we could mount *in vitro* efficient CD8+ T cell responses against neo-antigens derived from FSMs present in their tumor.

Altogether, we describe, for the first time to our knowledge, the precise immune signature of MSI-CRCs, and our results pave the way for developing personalized immunotherapy strategies in these cancers, especially for young Lynch patients.

## C14.3

**Studying the functionality of the homologous repair pathway in zebrafish embryos: heading for an *in vivo* functional test to evaluate the pathogenicity of BRCA2 variants identified in breast/ovarian cancer patients**

*K. B. M. Claes<sup>1</sup>, J. Vierstraete<sup>1</sup>, A. Willaert<sup>1</sup>, K. Vleminckx<sup>1</sup>, P. Vermassen<sup>1</sup>, P. Coucke<sup>1</sup>, A. Vral<sup>2</sup>;*

<sup>1</sup>*Center for Medical Genetics, Ghent University Hospital, Gent, Belgium, <sup>2</sup>Dept Basic Medical Sciences, Ghent University, Gent, Belgium.*

**Aims:** Currently, one of the major challenges in genetic testing is determining the functional effect of (rare) variants. Here, we propose a novel *in vivo* approach to study the functionality of BRCA2 missense variants in zebrafish. We propose an *in vivo* functional assay to measure in embryos the capacity of homologous recombination for human BRCA2 mRNA containing variants of unknown clinical significance (VUS).

**Methods:** We create DNA double strand breaks (DSB) in zebrafish embryos by irradiation. We use yH2AX and RAD51 foci assays as markers for DSB and HR repair respectively. We generated zebrafish brca2 knockdown models by morpholino injection and Crispr-Cas9. After synthesis of human BRCA2 mRNA, rescue experiments will be performed with wild type mRNA and mRNA containing the VUS of interest.

**Results:** We developed a protocol for visualising and quantifying RAD51 foci in tissues of wild type zebrafish embryos. Knockdown of brca2 by a morpholino results in almost complete absence of RAD51 foci in irradiated embryos. Similar results are currently being generated in the Crispr-Cas9 brca2 knockout model. In a next step we will rescue the phenotype by microinjection of wild type human BRCA2 mRNA and mRNA containing VUS to study the effect of these VUS on the HR capacity.

**Conclusions:** The zebrafish genome contains nearly all the genes involved in different DNA repair pathways in eukaryotes, including, homologous recombination (HR), in which BRCA2 plays a major role. Therefore, zebrafish provide an ideal *in vivo* model for studying variants in genes involved in DNA damage and repair.



## C14.4

### Cancer treatments contribute to the development of multiple primary tumours in Li-Fraumeni syndrome

*E. Kasper<sup>1</sup>, Y. Zerdoumi<sup>1</sup>, E. Colasse<sup>2</sup>, E. Angot<sup>2</sup>, L. Nicol<sup>3</sup>, S. Adriouch<sup>4</sup>, Y. Lacoume<sup>5</sup>, J. Sabourin<sup>2</sup>, T. Frebourg<sup>1</sup>, G. Bougeard<sup>1</sup>, J. Flaman<sup>1</sup>;*

<sup>1</sup>*Inserm U1079, Rouen University and Department of Genetics, Rouen University Hospital, Normandy Centre for Genomics and Personalized Medicine, Rouen, France,*

<sup>2</sup>*Inserm U1079, Rouen University and Department of Pathology, Rouen University Hospital, Normandy Centre for Genomics and Personalized Medicine, Rouen, France,*

<sup>3</sup>*Small Animal Magnetic Resonance Imaging Facility, Rouen University, Rouen, France,*

<sup>4</sup>*Inserm U905, Rouen University, Rouen, France, <sup>5</sup>Animal Facility, Rouen University, Rouen, France.*

Li-Fraumeni syndrome, due to TP53 germline mutations, is characterized by an exceptional high risk of multiple primary tumours. Considering that p53 pathway is activated in response to DNA damage, we recently developed an universal genotoxicity assay based on the transcriptional induction of TP53 target genes in human lymphocytes exposed to DNA damaging agents (Zerdoumi et al., *Mutat Res* 2015). To evaluate the impact of genotoxic agents in vivo, we first characterized the mouse transcriptome in response to DNA damage, selected Trp53 target genes, developed a similar genotoxic assay in mice based on the ex vivo exposure of mouse splenocytes and then directly performed the assay after intraperitoneal (IP) injection of chemotherapies. The mouse ex vivo and in vivo genotoxic assays showed, like the assay performed in human lymphocytes, that all classical chemotherapies, except microtubule poisons, are strongly genotoxic. We then submitted 6 weeks old Trp53 -/- LFS mice and Trp53 wt/wt control mice to IP injection of chemotherapies or radiotherapy and performed monthly total body MRI. We found that genotoxic chemotherapies and radiotherapy accelerate the development of malignant solid tumours and hemopathies in Trp53 -/- mice. All these results strongly support that cancer treatments contribute to the development of secondary tumours in LFS and explain the sequential occurrence of tumours observed in LFS patients. This indicates that, in LFS patients, radiotherapy should be avoided, whenever possible, and that non genotoxic chemotherapies should be evaluated in order to reduce the risk of multiple primary cancers.

## C14.5

### Single-cell transcriptional profiling identifies rare cell types with gene markers for classification and prognosis in Acute Myeloid Leukemia

*A. Sartori<sup>1</sup>, E. Falconnier<sup>1</sup>, P. Ribaux<sup>1</sup>, S. E. Antonarakis<sup>1,2,3</sup>, T. Matthes<sup>2</sup>, C. Borel<sup>1</sup>;*

<sup>1</sup>*University of Geneva Medical School, Geneva, Switzerland, <sup>2</sup>University Hospitals of Geneva, Geneva, Switzerland, <sup>3</sup>iGE3 Institute of Genetics and Genomics of Geneva, Geneva, Switzerland.*

Acute myeloid leukemia (AML) is a particularly aggressive blood cancer difficult to treat because of its recurrence affecting differentiation of hematopoietic progenitors. It is hypothesized that relapse of AML is due to incomplete eradication of leukemic stem cells (LSC) with self-renewal and leukemia-initiating properties. Our ultimate goal is to characterize LSC based on their transcriptional profiling.

Evidence suggests that CD34+CD38- cell population is enriched for LSCs but they remain rare and difficult to identify. Thus, we present a single-cell RNA-seq approach to characterize CD34+CD38- sorted cells in 2 AML individuals (AML1(M0), AML2(M5)) at the time of diagnosis and in 4 unaffected individuals (N). For 311 single-cells, 1764 genes were detected on average (RPKM>10). We identified 763 and 858 significantly differentially expressed genes in AML2 and AML1, respectively using D3E. We demonstrate that the captured AML cells possess the hallmarks of LSC. The top highly expressed genes are stem markers including SOX4, CD74, CD69 and CXCL8. In particular, HOXA-B cluster genes appeared markedly dysregulated demonstrating their leukemia-initiating capability. Gene ontology revealed enrichment for annotation of apoptotic resistance and self-renewal capacity as well. Furthermore, 3 distinct clusters of co-expressed genes were identified that distinguished between N cells and AML cells with M0 and M5 (SEURAT-tool). Finally, we hypothesized that prognostic gene-expression signature is present at diagnosis. Using TCGA RNA-seq and clinical datasets, we identified genes whose expression correlated with AML patient survival. We confirmed their differential expression in AML single-cells and thus, their contribution in leukemogenesis and potential relevance for clinical-outcome prediction.

## C14.6

### Tracing the origin of disseminated tumor cells in breast cancer using single-[[Unsupported Character - Codename &shy;]cell sequencing

*P. Kumar<sup>1,2</sup>, E. Moller<sup>3,4</sup>, J. Demeulemeester<sup>1,5</sup>, S. Nord<sup>3,4</sup>, D. Wedge<sup>2</sup>, M. Zamani Esteki<sup>1</sup>, A. Børresen-Dale<sup>3</sup>, K. White<sup>6</sup>, B. Naume<sup>3</sup>, V. Kristensen<sup>3</sup>, P. Van Loo<sup>5,2</sup>, T. Voet<sup>1,2</sup>,*

<sup>1</sup>*University of Leuven, Leuven, Belgium, <sup>2</sup>Welcome Trust Sanger Institute, Hinxton, United Kingdom, <sup>3</sup>University of Oslo, Oslo, Norway, <sup>4</sup>Institute for Cancer Research, Oslo, Norway, <sup>5</sup>The Francis Crick Institute, London, United Kingdom, <sup>6</sup>University of Chicago, Chicago, IL, United States.*

Single-cell micro-metastases of solid tumors often occur in bone marrow. These disseminated tumor cells (DTCs) may lay dormant, resist therapy and may cause overt bone and visceral metastases. Unfortunately, the molecular nature of DTCs remains elusive, as well as when and where they spring from the tumor. Here, we apply single-cell sequencing to identify and trace the origin of DTCs in breast cancer.

Results: We sequenced 40 single cells isolated by micromanipulation from the bone marrow of six patients using established immunocytochemical markers and morphologic characteristics for epithelial tumor cells. Comparison of the cells' DNA copy number (CN) profiles with those of the primary tumors and lymph node metastasis established that a quarter of the cells disseminated from a tumor clone. The remaining cells represented non-aberrant 'normal' cells and 'aberrant cells of unknown origin' that have CN landscapes discordant from the tumor. Genotyping somatic mutations called on bulk tumor exomes in the SC sequences confirmed that these latter cells did not derive from the same lineage as the breast cancers. Evolutionary reconstruction analysis of bulk tumor and DTC genomes enabled ordering of CNA events in molecular pseudo-time and tracing the origin of the DTCs to either the main tumor clone, primary tumor subclones, or subclones in lymph node metastases.

Conclusions: SC sequencing, in parallel with intra-tumor genetic heterogeneity profiling from bulk DNA, is a powerful approach to identify and study DTCs, yielding insight into metastatic processes. A heterogeneous population of CNA-positive cells of unknown origin is prominent in bone marrow.

## C15 Neurogenetic disorders

### C15.1

#### A Complex Mutational Spectrum of Structural Variation Contributing to Autism

*M. E. Talkowski<sup>1,2,3</sup>, R. Collins<sup>4</sup>, C. Redin<sup>1</sup>, M. Stone<sup>1</sup>, J. Glessner<sup>1</sup>, S. Sanders<sup>4</sup>, H. Brand<sup>1,2</sup>,*

<sup>1</sup>*Massachusetts General Hospital, Boston, MA, United States, <sup>2</sup>Harvard Medical School, Boston, MA, United States, <sup>3</sup>Broad Institute, Cambridge, MA, United States, <sup>4</sup>University of California San Francisco, San Francisco, CA, United States.*

Recent genomic studies of autism spectrum disorder (ASD) have relied upon genome-wide characterization of coding mutations using whole-exome sequencing (WES) and copy number variants (CNV) using chromosomal microarray (CMA). In ASD, these efforts have revealed a strong association with de novo loss-of-function (LoF) mutations and CNVs segments that alter the dosage of genes under evolutionary constraint. Unfortunately, the impact of balanced and complex structural variation (SV), which also represent LoF variation, remains uncharacterized in ASD and other human disease studies. Here, we explored the full mutational spectrum of SV contributing to ASD by integrating large-insert, short-insert and synthetic long-read whole-genome sequencing (WGS) technologies with CMA in 307 idiopathic ASD probands and 120 family members from the Simons Simplex Collection. Analyses revealed a diverse landscape of SVs beyond canonical CNVs, including inversions, insertions, and translocations. We also categorized 13 distinct subclasses of recurrent complex SVs and demonstrate that each genome sequenced harbored at least one such large complex rearrangement (~5 kb resolution). We identified 26 de novo SVs likely contributing to ASD, 50% of which were cryptic to detection by previous technologies on these same samples. These data confirmed 13 previously proposed ASD risk factors and discovered 12 novel candidates. We estimate that the overall contribution of de novo SVs in ASD at this resolution to be at least 8.2%, or a two-fold increase over previous analyses restricted to CNVs. These data suggest that cryptic and complex SV represent an important and presently underappreciated component of ASD etiology.


**C15.2**

### A potential role for the linker for activation of T-cells (LAT) in the neuroanatomical phenotype of the 220kb 16p11.2 BP2-BP3 CNVs

*M. Loviglio<sup>1</sup>, T. Arbogast<sup>2</sup>, M. Leleu<sup>3</sup>, G. Giannuzzi<sup>4</sup>, K. Männik<sup>1</sup>, 16p11.2 Consortium, J. S. Beckmann<sup>5</sup>, S. Jacquemont<sup>6</sup>, N. Katsanis<sup>2</sup>, C. Golzio<sup>2</sup>, A. Reymond<sup>1</sup>;*

<sup>1</sup>Center for Integrative Genomics, Lausanne, Switzerland, <sup>2</sup>Center for Human Disease Modeling - Duke University, Durham, NC, United States, <sup>3</sup>EPFL, Lausanne, Switzerland,

<sup>4</sup>Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland, <sup>5</sup>CHUSJ - Université de Montréal, Montréal, QC, Canada.

Copy number variants (CNVs) of the distal 16p11.2 220kb BP2-BP3 region show mirror effect on BMI and head size, and association with autism spectrum disorders (ASD) and schizophrenia, as previously reported for the proximal 16p11.2 600kb BP4-BP5 deletion and duplication. These two CNVs-prone regions at 16p11.2 are also reciprocally engaged in complex chromatin looping, successfully confirmed by 4C, FISH, Hi-C and concomitant expression changes. We assessed the 220kb BP2-BP3 duplication by overexpressing each of the 9 encompassed human transcripts, CD19, NFATC2IP, ATXN2L, TUFM, ATP2A1, RABEP2, SPNS1, LAT and SH2B1, in zebrafish embryos. Overexpression of the linker for activation of T cells (LAT) induced a reduction in dividing cells in the brain and number of post-mitotic neurons in the anterior forebrain at 2dpf, and of intertectal axonal tracts at 3dpf, resulting in microcephaly at later stages. We showed similar results upon overexpression of CD247 and ZAP70, two genes belonging to the LAT's immune system signaling pathway. Co-injections experiments showed that KCTD13, MVP, and MAPK3, three genes mapping within the 600kb BP4-BP5 locus and major driver and modifiers, respectively, of the head circumference phenotype linked to that region, and LAT acted in an additive manner to increase the severity of the microcephaly phenotype, suggesting the presence of genetic interaction. Chromatin conformation capture further showed that LAT cis- and trans-interacting chromatin partners were enriched for ASD genes (p=5.6E-03, OR=1.9). Our results suggest that LAT, besides its well-recognized function in T-cells development, is a major contributor in the 16p11.2 (BP2-BP3) 220kb CNVs neurodevelopmental phenotypes.


**C15.3**

### Disruption of POGZ is associated with intellectual disability and autism spectrum disorders

*H. A. F. Stessman<sup>1</sup>, M. H. Willemsen<sup>2</sup>, M. Fenckova<sup>2</sup>, O. Penn<sup>1</sup>, A. Hoischen<sup>2</sup>, B. Xiong<sup>1</sup>, T. Wang<sup>3</sup>, K. Hoekzema<sup>1</sup>, L. Vives<sup>4</sup>, I. Vogel<sup>4</sup>, H. G. Brunner<sup>5</sup>, I. van der Burgt<sup>2</sup>, C. Ockelen<sup>2</sup>, J. Schuurs-Hoeijmakers<sup>2</sup>, J. Klein Wassink-Ruitter<sup>5</sup>, C. Stumpel<sup>6</sup>, J. Vles<sup>6</sup>, C. Marcelis<sup>2</sup>, H. van Bokhoven<sup>2</sup>, V. Cantagrel<sup>7</sup>, S. Lyonnnet<sup>7</sup>, L. Colleaux<sup>7</sup>, R. Bernier<sup>1</sup>, J. Gerdt<sup>8</sup>, B. P. Coe<sup>1</sup>, C. Romano<sup>9</sup>, L. Grillo<sup>9</sup>, A. Albert<sup>9</sup>, C. Scuderi<sup>10</sup>, M. Nordenskjöld<sup>10</sup>, M. Kvärnung<sup>10</sup>, H. Guo<sup>11</sup>, K. Xia<sup>11</sup>, A. Piton<sup>12,13</sup>, B. Gerard<sup>13</sup>, D. Genevieve<sup>14</sup>, B. Delobel<sup>15</sup>, D. Lehal<sup>16</sup>, L. Perrin<sup>17</sup>, F. Prieur<sup>18</sup>, J. Thevenon<sup>16</sup>, J. Geczi<sup>19</sup>, M. Shaw<sup>19</sup>, R. Pfundt<sup>2</sup>, B. Keren<sup>20</sup>, A. Jacquette<sup>20</sup>, A. Schenck<sup>2</sup>, E. Eichler<sup>1,21</sup>, T. Kleefstra<sup>2</sup>;*

<sup>1</sup>University of Washington, Seattle, WA, United States, <sup>2</sup>Radboud University Medical Centre, Nijmegen, Netherlands, <sup>3</sup>School of Life Sciences, Changsha, China, <sup>4</sup>Aarhus University Hospital, Aarhus, Denmark, <sup>5</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>6</sup>Maastricht UMC+, Maastricht, Netherlands, <sup>7</sup>Institut Imagine, INSERM, Paris, France, <sup>8</sup>IRCCS Associazione Oasi Maria Santissima, Troina, Italy, <sup>9</sup>IRCCS Associazione Oasi Maria Santissima, Toina, Italy, <sup>10</sup>Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, <sup>11</sup>School of Life Sciences, Central South University, Changsha, China, <sup>12</sup>Institut Génétique Biologie Moléculaire Cellulaire, Illkirch, France, <sup>13</sup>Hôpitaux Universitaires de Strasbourg, Strasbourg, France, <sup>14</sup>Hôpital Arnaud de Villeneuve, Montpellier, France, <sup>15</sup>Hôpital Saint Vincent de Paul, Lille, France, <sup>16</sup>Fédération Hospitalo-Universitaire TRANSLAD, Dijon, France, <sup>17</sup>Hôpital Robert Debré, Paris, France, <sup>18</sup>CHU de Saint-Étienne, Saint-Étienne, France, <sup>19</sup>Robinson Research Institute and the University of Adelaide at the Women's and Children's Hospital, North Adelaide, Australia, <sup>20</sup>Hôpital de la Pitié-Salpêtrière, Paris, France, <sup>21</sup>Howard Hughes Medical Institute, Seattle, WA, United States.

**Introduction:** Intellectual disability (ID) and autism spectrum disorders (ASD) are genetically heterogeneous, and associated genes for both conditions overlap substantially. Few mutations in POGZ were reported in recent exome studies. However, these studies did not provide detailed clinical information, leaving the pathogenicity and a clear phenotypic definition of the POGZ patient unknown.

**Patients and Methods:** We collected the clinical and molecular data of 25 individuals with disruptive mutations in POGZ identified by diagnostic whole-exome, whole-genome, or targeted sequencing of 5,223 individuals with neurodevelopmental disorders (ID primarily) or by targeted resequencing of this locus in 12,041 individuals with ASD and/or ID. In parallel, we utilized a *Drosophila* knockdown model of the POGZ orthologue *row* to provide further support for the importance of POGZ in cognitive function.

**Results:** We defined common phenotypic features of POGZ patients, including variable levels of developmental delay and more severe speech and language delay compared to motor delay and coordination issues. We also identified significant associations with vision problems, microcephaly, hyperactivity, a tendency to obesity and feeding difficulties. In addition, we

found that downregulation of *row* expression, specifically in neurons, leads to deficits in habituation, a form of non-associative learning that is highly relevant for both ID and ASD.

**Conclusion:** Combined, these data underscore the pathogenicity of loss-of-function mutations in POGZ and define a novel POGZ-related syndrome. Some features may be explained by the high expression of POGZ early in fetal brain development, particularly in the cerebellum and pituitary.


**C15.4**

### CLOZUK2: A population-based approach to the genetics of treatment-resistant schizophrenia

*A. F. Pardiñas<sup>1</sup>, S. Legge<sup>1</sup>, S. Ripke<sup>2</sup>, the CRESTAR Consortium, K. D. Chambert<sup>3</sup>, J. L. Moran<sup>3</sup>, S. A. McCarroll<sup>3</sup>, M. J. Owen<sup>4</sup>, M. C. O'Donovan<sup>1</sup>, J. T. R. Walters<sup>5</sup>;*

<sup>1</sup>MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, Cardiff, United Kingdom, <sup>2</sup>Universitätsmedizin Berlin: Charité, Berlin, Germany, <sup>3</sup>Stanley Center for Psychiatric Research, The Broad Institute of MIT and Harvard, Cambridge, MA, United States.

About one third of patients with schizophrenia do not respond adequately to antipsychotic medication, and are thus considered to have treatment-resistant schizophrenia (TRS). This is one of the most disabling forms of mental illness and constitutes a major challenge and cost to public health, affecting at least 1 million people solely in Europe. The only drug backed by evidence-based studies as a prescription for TRS is the atypical antipsychotic clozapine, which requires frequent blood monitoring due to rare but potentially lethal adverse effects.

Through an agreement with the major pharmaceutical companies that market clozapine in the UK (Novartis and Leyden Delta) we collected blood samples of 15,210 anonymous clozapine takers, which were genotyped on the Illumina OmniExpress beadchip by the Broad Institute of MIT and Harvard (Cambridge, USA) and deCODE genetics (Reykjavík, Iceland). We obtained genotype data from 25,811 unscreened control individuals through public sources and collaboration with large-scale genotyping projects. Analysis of this dataset showed significant associations in known schizophrenia loci (TSNARE1, CACNA1C, DRD2) and novel signals (RAPGEF4, ZNF664) which have been previously linked to brain development. In order to clarify the relationship between broad-sense schizophrenia and TRS, we generated polygenic risk prediction scores from the largest available schizophrenia GWAS (Ripke et al. 2015), which were used to calculate risk profiles in TRS individuals. There was no compelling evidence of a stronger polygenic signal in this analysis, disputing the view that TRS represents simply a severe form of schizophrenia.

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**C15.5**

### H-prune is required for microtubule assembly and is mutated in microcephaly and neurodevelopmental delay

*M. Y. Ahmed<sup>1,2</sup>, E. Baple<sup>3,4</sup>, M. Carotenuto<sup>5,6</sup>, R. Singh<sup>7</sup>, B. Chioza<sup>3</sup>, P. De Antonellis<sup>5,6</sup>, V. Ferrucci<sup>5,6</sup>, R. N. Chaurasia<sup>7</sup>, T. M. Mohapatra<sup>8</sup>, R. Prasad<sup>9</sup>, P. Pucci<sup>6</sup>, R. Rashmi<sup>7</sup>, F. Cozzolino<sup>6</sup>, R. K. Sunil<sup>7</sup>, M. Monti<sup>6</sup>, A. Rajab<sup>9</sup>, A. Al-Amri<sup>9</sup>, A. Alshehi<sup>10</sup>, M. Patton<sup>1,2</sup>, M. Zollo<sup>5,6</sup>, A. H. Crosby<sup>1,3</sup>;*

<sup>1</sup>Genetics Research Centre, St. George's University London, London, United Kingdom,

<sup>2</sup>RILD Wellcome Wolfson Centre, Royal Devon & Exeter NHS Foundation Trust, Exeter, United Kingdom, <sup>3</sup>Medical Research (Level 4), RILD Wellcome Wolfson Centre, Royal

Devon & Exeter NHS Foundation, Exeter, United Kingdom, <sup>4</sup>University Hospital

Southampton NHS Foundation Trust, Wessex Clinical Genetics Service, Southampton, United Kingdom, <sup>5</sup>Dipartimento di Biochimica e Biotecnologie Mediche, Università degli

Studi di Napoli Federico II, Naples, Italy, <sup>6</sup>CEINGE Biotecnologie Avanzate, Naples, Italy,

<sup>7</sup>Molecular Genetics, Department Of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, <sup>8</sup>Department of Pediatrics, Institute of Medical Sciences Banaras Hindu University, Varanasi, India, <sup>9</sup>National Genetic Centre, Ministry of Health, Muscat, Oman, <sup>10</sup>Department of Biology, Sultan Qaboos University, Muscat, Oman.

**Introduction:** Microtubules are crucial cytoplasmic structures regulating neuronal cells migration and polarity which are key processes in brain morphogenesis. Their important role in human brain development is highlighted by the neurological disorders arising from mutations in genes encoding tubulins or their cellular interacting partners.

**Materials and methods:** Using genome wide SNP genotyping paralleled with whole exome sequencing, we investigated a large multigenerational Omani pedigree and a single nuclear family from India to identify the genetic basis of a severe autosomal recessive neurodevelopmental condition with cardinal features of microcephaly and global developmental delay. We performed in vitro cell assays to examine the functional consequences of the identified mutations in key cellular processes.

**Results:** We identified two novel mutations in the PRUNE gene affecting key functional motifs of the encoded protein: (c.88G>A/ p.Asp30Asn) in the

extended Omani family, and (c.889C>T/ p.Arg297Trp) in the Indian family. Our studies identified PRUNE as a binding partner of  $\beta$ -tubulin crucial for tubulin polymerization. This interaction is impaired by both mutations as shown by disrupted subcellular co-localizations and undermined in vitro microtubules assembly. We also show that both mutations impair in vitro cell migration, proliferation and differentiation which are processes normally enhanced by wild type PRUNE.

Conclusions: Our data confirm the essential role of tubulin-related proteins in cortical development and define PRUNE as a pivotal interacting molecule important for normal microtubule assembly which may be mutated to result in human microcephaly and a global neurodevelopmental delay phenotype. Supported by MRC grant: G1002279

## C15.6

### Primary microcephaly: ALFY-controlled DVL3 autophagy regulates Wnt signaling, determining human brain size

R. Kadir<sup>1</sup>, T. Harel<sup>1</sup>, B. Markus<sup>1</sup>, Y. Perez<sup>1</sup>, A. Bakhrat<sup>2</sup>, E. Chervinski<sup>3</sup>, J. Zlotogora<sup>4</sup>, S. Sivan<sup>5</sup>, R. Y. Birnbaum<sup>2</sup>, U. Abdu<sup>2</sup>, S. Shalev<sup>3</sup>, O. S. Birk<sup>5,6</sup>;

<sup>1</sup>Human Genetics and the Genetics Institute, Soroka Medical Center, Ben-Gurion University, Beer Sheva, Israel, <sup>2</sup>Department of Life Sciences, Ben Gurion University, Beer Sheva, Israel, <sup>3</sup>Genetics Institute, HaEmek Medical Center, Afula, Israel, <sup>4</sup>Department of Community Genetics, Public Health Services, Ministry of Health, Jerusalem, Israel, <sup>5</sup>The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev and Faculty of Health Sciences, Ben Gurion University, Beer Sheva, Israel, <sup>6</sup>Genetics Institute, Soroka University Medical Center, Ben Gurion University, Beer Sheva, Israel.

Primary microcephaly is a congenital neurodevelopmental disorder of reduced head circumference and brain volume, with fewer neurons in the cortex of the developing brain due to premature transition between symmetrical and asymmetrical cellular division of the neuronal stem cell layer during neurogenesis. We now show through linkage analysis and whole exome sequencing, that a dominant mutation in ALFY, encoding an autophagy scaffold protein, causes human primary microcephaly. We demonstrate the dominant effect of the mutation in drosophila: transgenic flies harboring the human mutant allele display small brain volume, recapitulating the disease phenotype. Moreover, eye-specific expression of human mutant ALFY causes rough eye phenotype. In molecular terms, we demonstrate that normally ALFY attenuates the canonical Wnt signaling pathway via autophagy-dependent removal specifically of aggregates of DVL3 and not of Dvl1 or Dvl2. Thus, autophagic attenuation of Wnt signaling through removal of Dvl3 aggregates by ALFY acts in determining human brain size.

## C16 Bioinformatics, statistical and population genetics

### C16.1

#### FINEMAP: Ultrafast high-resolution fine-mapping using summary data from genome-wide association studies

C. Benner<sup>1,2</sup>, C. Spencer<sup>3</sup>, A. S. Havulinna<sup>4</sup>, V. Salomaa<sup>4</sup>, S. Ripatti<sup>1,2,5</sup>, M. Pirinen<sup>1</sup>;  
<sup>1</sup>Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland, <sup>2</sup>Department of Public Health, University of Helsinki, Helsinki, Finland, <sup>3</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, <sup>4</sup>National Institute for Health and Welfare (THL), Helsinki, Finland, <sup>5</sup>Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom.

Genome-Wide Association Studies (GWAS) have identified thousands of loci associated with complex diseases. A next crucial step is fine-mapping: identifying causal variants that point to molecular mechanisms behind the associations and, eventually, suggest therapeutic targets. Recently, fine-mapping methods have been extended to use only GWAS summary data together with pairwise correlations of the variants. Common to these approaches is that they rely on computationally expensive exhaustive search restricting their use to a few hundred variants. We introduce a software package FINEMAP that replaces the exhaustive search by an ultrafast stochastic search and thereby allows fine-mapping analyses to scale up to whole chromosomes.

We show that FINEMAP (1) opens up completely new opportunities by, e.g., exploring 15q21/LIPC locus for HDL-cholesterol with 20,000 variants in less than 90 seconds while exhaustive search would require more than 9,000 years, (2) provides similar accuracy to exhaustive search when the latter can be completed, (3) achieves even higher accuracy when the latter must be restricted due to computational reasons, and (4) identifies more plausible variant combinations than conditional analysis. At 15q21/LIPC locus with at least a 3-SNP association pattern with HDL, a missense variant and a promoter polymorphism are likely to be causal whereas the lead

variant in single-SNP testing has less evidence than a regulatory variant correlated with it.

We believe that FINEMAP's approach of jointly modeling the whole locus together with its unprecedented computational efficiency will help reveal valuable knowledge that could otherwise remain hidden due to limitations of existing fine-mapping methods.

### C16.2

#### Phenotype similarity regression for identifying the genetic determinants of rare diseases

E. Turro<sup>1</sup>, D. Greene<sup>1</sup>, S. Richardson<sup>2</sup>, NIHR BioResource;

<sup>1</sup>University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>MRC Biostatistics Unit, Cambridge, United Kingdom.

Rare genetic disorders, which can now be studied systematically with affordable genome sequencing, are often caused by high-penetrance rare variants. Such disorders are often heterogeneous and characterised by abnormalities spanning multiple organ systems ascertained with variable clinical precision. Existing methods for identifying genes with variants responsible for rare diseases summarise phenotypes with unstructured binary or quantitative variables. The Human Phenotype Ontology (HPO) allows composite phenotypes to be represented systematically but association methods accounting for the ontological relationship between HPO terms do not exist. We present a Bayesian method to model the association between an HPO-coded patient phenotype and genotype. Our method estimates the probability of an association together with an HPO-coded phenotype characteristic of the disease. We thus formalise a clinical approach to phenotyping that is lacking in standard regression techniques for rare disease research. We demonstrate the power of our method by uncovering a number of true associations in a large collection of genome-sequenced and HPO-coded cases with rare diseases.

### C16.3

#### PRSlice: A new polygenic risk score approach that leverages pleiotropy to improve prediction of complex traits

J. Euesden, Y. Ruan, A. Socrates, J. R. I. Coleman, C. M. Lewis, P. F. O'Reilly; King's College London, London, United Kingdom.

Polygenic Risk Scores (PRS) are widely applied in human disease genetics. We have developed the first dedicated software for calculating, testing and plotting the results of polygenic risk scores - PRSlice ('precise'). To improve PRS predictive power further, we investigate an alternative approach to the selection of SNPs to calculate genetic risk, in particular in the context of applying PRS to assess shared genetic aetiology between different traits. By splitting the genome into chunks (eg. 5Mb regions) and selecting an optimum threshold for each chunk, we are able to select SNPs with large effects on the target phenotype but more modest effects on the discovery phenotype, which would have been omitted based on discovery P-value alone. We demonstrate, via application to real and simulated data, that this novel method - PRSlice - can improve phenotype prediction across a number of common contingencies. We firstly focus on using summary data for one disease to predict risk of another comorbid phenotype - this not only improves disease prediction in scenarios where the highest powered GWAS available is in a different but related trait, such as using BMI genetic risk to predict hypertension - but also provides insight into the shared genetic architecture between complex traits. Secondly, we demonstrate the value of this approach for predicting genetic risk across different worldwide populations, an increasingly common analysis strategy, demonstrating that the effects of allelic heterogeneity can be accounted for.

### C16.4

#### Emergence of a *Homo sapiens*-specific gene family and the evolution of disease risk at chromosome 16p11.2

G. Giannuzzi<sup>1</sup>, X. Nuttle<sup>2</sup>, M. H. Duyzend<sup>2</sup>, J. G. Schraiber<sup>2</sup>, I. Narvaiza<sup>3</sup>, F. Camponeschi<sup>4</sup>, P. Sudmant<sup>2,5</sup>, O. Penn<sup>2</sup>, G. Chiatante<sup>2</sup>, M. Malig<sup>2</sup>, J. Huddleston<sup>2,7</sup>, C. Benner<sup>3</sup>, M. C. N. Marchetto<sup>3</sup>, L. Denman<sup>2</sup>, L. Harshman<sup>2</sup>, C. Baker<sup>2</sup>, A. Raju<sup>2,7</sup>, K. Penewitt<sup>2</sup>, N. Janke<sup>2</sup>, J. W. Tang<sup>8</sup>, M. Ventura<sup>6</sup>, F. Antonacci<sup>2</sup>, L. Banci<sup>4,9</sup>, S. Ciofi-Baffoni<sup>4,9</sup>, J. M. Akey<sup>2</sup>, C. T. Amemiya<sup>8</sup>, F. H. Gage<sup>2,10</sup>, A. Reymond<sup>1</sup>, E. E. Eichler<sup>2,7</sup>;

<sup>1</sup>Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland,

<sup>2</sup>Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA, United States, <sup>3</sup>Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA, United States, <sup>4</sup>Department of Chemistry, University of Florence, Florence, Italy, <sup>5</sup>Program in Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA, United States, <sup>6</sup>Department of Biology, University of Bari, Bari, Italy, <sup>7</sup>Howard Hughes Medical Institute, Seattle, WA, United States, <sup>8</sup>Benaroya Research Institute at Virginia Mason, Seattle, WA, United States, <sup>9</sup>Magnetic Resonance

Center CERM, University of Florence, Florence, Italy, <sup>10</sup>Center for Academic Research and Training in Anthropogeny, La Jolla, CA, United States.

Recurrent pathogenic deletions and duplications at chromosome 16p11.2 are mediated by a complex set of highly identical and directly oriented segmental duplications. This disease-predisposing architecture results from recent, *Homo sapiens*-specific duplications (i.e. absent in Neanderthal and Denisovan) of a segment including the *BOLA2* gene, the latest among a series of genomic changes that dramatically restructured the region during hominid evolution. All humans examined (n = 2,359) carry one or more copies of the duplication, which nearly fixed early in the human lineage—a pattern unlikely to have arisen so rapidly in the absence of selection (p < 0.009). We show that *BOLA2* duplications affect its expression levels among individuals and between humans and other primates. Its copy number correlates with both RNA expression ( $r = 0.36$ ) and protein level ( $r = 0.65$ ) in human lymphoblastoid cell lines. *BOLA2* is highly expressed in embryonic stem cells, where it shows the greatest expression difference between human and chimpanzee. *BOLA2* localizes to the cell cortex and forms a [2Fe-2S]-bridged heterotrimeric complex with *GLRX3* (glutaredoxin 3). We show that *in vitro* increasing *BOLA2* results in a shift from dimeric *GLRX3* to heterotrimeric *BOLA2/GLRX3* complex and the latter is 3-fold more stable upon air exposure. We are currently investigating the cellular phenotypes associated with *BOLA2* differential expression and oxidative stress conditions to gain insights into the possible advantage linked to the emergence of the *BOLA2* gene family at the root of the *Homo sapiens* lineage.

Chile, <sup>6</sup>Departamento de Genética, Universidad Federal do Rio Grande do Sul, Porto Alegre, Brazil, <sup>7</sup>Laboratorio de Genética Molecular, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellin, Colombia, <sup>8</sup>Centro Nacional Patagónico, CONICET, Puerto Madryn, Argentina.

Latin America has a complex history of extensive admixture between Native Americans, Europeans and Africans. Thus far, this process has been explored with genetic data to examine patterns of continental ancestry. Here we explore sub-continental ancestry using ~700,000 autosomal SNPs in a sample of ~7,000 Latin Americans from Brazil, Chile, Colombia, Mexico and Peru (DOI: 10.1371/journal.pgen.1004572).

We describe a novel statistical approach exploiting patterns of haplotype similarity with increased precision over a related approach that was applied successfully to study the fine scale patterns of worldwide populations (DOI: 10.1126/science.1243518) and Great Britain (DOI: 10.1038/nature14230). We identify the contributions of geographically precise ancestral components, including those from Africa and Europe, to both regions and individuals. For Brazil, we highlight how the sources of European ancestry vary considerably, reflecting influx at different times from the Iberian Peninsula, Italy and Northern Europe. In the former Spanish colonies, we identify ancestry from specific regions in Spain at the individual level. Strikingly, Native American ancestry within Latin Americans shows a strong correlation with DNA patterns of specific local Native groups. We demonstrate that genetic patterns among these Native groups, as well as among different migrant groups, are clearly differentiated, which has important implications in understanding how genetic associations to phenotypes vary across Latin Americans with widely heterogeneous ancestral backgrounds. Overall, we are able to identify ancestry patterns at higher resolution than has been previously achieved, allowing us a fine grained analysis of how history shaped the genetic makeup of Latin America and how this affects association studies.

## C16.5

### Using whole exome sequence based imputation panel to boost considerably the number of successfully imputed low-frequency and rare coding variants in the Finnish founder population

*I. Surakka*<sup>1,2</sup>, *A. Sarin*<sup>1,2</sup>, *K. Karczewski*<sup>3,4</sup>, *R. Durbin*<sup>5</sup>, *D. MacArthur*<sup>3,4</sup>, *V. Salomaa*<sup>2</sup>, *A. Palotie*<sup>1,4,6</sup>, *S. Ripatti*<sup>1,5,7</sup>, *SISu project group*

<sup>1</sup>Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland, <sup>2</sup>National Institute for Health and Welfare, Helsinki, Finland, <sup>3</sup>Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, United States, <sup>4</sup>Program in Medical and Population Genetics, Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA, United States, <sup>5</sup>Wellcome Trust Sanger Institute, Hinxton, United Kingdom, <sup>6</sup>Psychiatric & Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Boston, MA, United States, <sup>7</sup>Department of Public Health, University of Helsinki, Helsinki, Finland.

It has been shown that population specific reference panel improves imputation accuracy especially in the case of rare and low-frequency variants. We studied how much better we can impute variants using a population based sequence reference panel instead of commonly used global 1000 Genomes or the Haplotype Reference Consortium (HRC) reference panels. Specifically, we evaluated the utility of extending the Finnish low-coverage whole genome sequencing (WGS) panel (N = 1,941) with a jointly called whole exome sequencing (WES) based reference panel (N = 4,932) to successfully impute coding variation into our test dataset of 10,489 Finns. Using the Finnish WGS reference panel we saw a ~100% increase in the number of well-imputed exonic variants in 0.1-0.5% allele frequency range compared to the 1000G panel. The HRC reference panel gave us 7% more variants compared to the Finnish WGS panel but also introduced 0.8% false positive variant calls compared to 0.1% with the Finnish WGS reference panel mostly driven by rare variants with allele frequency <0.1%. By combining the WES panel with the population specific WGS reference panel, we observed over 20% more well-imputed variants in the 0.1-0.5% allele frequency range compared to the Finnish WGS only panel. As an example, we successfully imputed 422 loss-of-function (LoF) variants enriched in the Finnish population into the test dataset. These variants are now the basis of an intense phenome-wide association (PheWAS) testing. Our results show that population specific WES based reference panel boost considerably the imputation of rare and low-frequency coding variants.

## C16.6

### Genetic history of Latin America: Fine-scale population structure and phenotypic diversity

*J. Chacón-Duque*<sup>1</sup>, *K. Adhikari*<sup>1</sup>, *V. Acuña-Alonso*<sup>2,1</sup>, *S. Canizales-Quinteros*<sup>3</sup>, *C. Gallo*<sup>4</sup>, *G. Poletti*<sup>4</sup>, *F. Rothhammer*<sup>5</sup>, *M. Bortolini*<sup>6</sup>, *G. Bedoya*<sup>7</sup>, *R. Gonzalez-José*<sup>6</sup>, *D. Balding*<sup>1</sup>, *CANDELA Consortium*, *G. Hellenthal*<sup>1</sup>, *A. Ruiz-Linares*<sup>1</sup>

<sup>1</sup>Department of Genetics, Evolution and Environment, University College London, London, United Kingdom, <sup>2</sup>National Institute of Anthropology and History, Mexico City, Mexico, <sup>3</sup>Facultad de Medicina and Facultad de Química, UNAM; Instituto Nacional de Ciencias Médicas y Nutrición 'Salvador Zubirán', Mexico City, Mexico, <sup>4</sup>Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Perúana Cayetano Heredia, Lima, Peru, <sup>5</sup>Instituto de Alta Investigación Universidad de Tarapacá, Programa de Genética Humana ICBM Facultad de Medicina Universidad de Chile, Arica,

## C17 Metabolic and mitochondrial disorders

### C17.1

#### Comprehensive genomic analyses of Japanese cases with mitochondrial respiratory chain complex deficiencies

*M. Kohda*<sup>1</sup>, *Y. Tokuzawa*<sup>1</sup>, *Y. Kishita*<sup>1</sup>, *Y. Moriyama*<sup>1</sup>, *Y. Mizuno*<sup>1</sup>, *T. Hirata*<sup>1</sup>, *Y. Yatsuka*<sup>1</sup>, *Y. Yamashita-Sugahara*<sup>1</sup>, *Y. Nakachi*<sup>1</sup>, *H. Kato*<sup>1</sup>, *S. Tamaru*<sup>1</sup>, *H. Nyuzuki*<sup>1</sup>, *N. N. Borna*<sup>1</sup>, *H. Harashima*<sup>1</sup>, *T. Yamazaki*<sup>1</sup>, *M. Mori*<sup>2</sup>, *K. Murayama*<sup>2</sup>, *A. Ohtake*<sup>1</sup>, *Y. Okazaki*<sup>1</sup>, <sup>1</sup>Saitama Medical University, Hidaka, Japan, <sup>2</sup>Matsudo City Hospital, Matsudo, Japan, <sup>3</sup>Chiba Children's Hospital, Chiba, Japan.

Mitochondrial disorders have the highest incidence among inborn errors of metabolism and are characterized by biochemical respiratory chain complex deficiencies. It occurs at a rate of 1 in 5,000 births, and has phenotypic and genetic heterogeneity. Mutations in about 1,500 nuclear encoded mitochondrial proteins may cause mitochondrial dysfunction of energy production and mitochondrial disorder. More than 250 genes that cause mitochondrial disorder have been reported to date. However exact genetic diagnosis for patients still remained largely unknown. To reveal this heterogeneity, we performed comprehensive genomic analyses for 142 patients with childhood-onset mitochondrial respiratory chain complex deficiencies. The approach includes whole mtDNA and exome analyses using high-throughput sequencing, and chromosomal aberration analyses using high-density oligonucleotide arrays. We identified 41 mutation in known mitochondrial disease genes, of which 37 were novel. We also identified and 4 mitochondrial-related genes (*MRPS23*, *QRSL1*, *SLC25A26* and *PNPLA4*) as novel causative genes. We further identified 2 genes known to cause monogenic diseases (*MECP2* and *TNNI3*) and 3 chromosomal aberrations (6q24.3-q25.1, 17p12, and 22q11.21) as causes in this cohort. Our approaches enhance the ability to identify pathogenic gene mutations in patients with biochemically defined mitochondrial respiratory chain complex deficiencies in clinical settings. They also underscore clinical and genetic heterogeneity of mitochondrial respiratory chain complex deficiencies and will improve patient care of this complex disorder.

## C17.2

**Recessive mutations in TRMT10C cause defects in mitochondrial RNA processing and multiple respiratory chain deficiencies**

*M. D. Metodieva<sup>1</sup>, K. Thompson<sup>2</sup>, C. L. Alston<sup>2</sup>, A. Morris<sup>3</sup>, L. He<sup>2</sup>, Z. Assouline<sup>4</sup>, M. Rio<sup>4</sup>, N. Bahi-Buisson<sup>4</sup>, A. Pyle<sup>5</sup>, H. Griffin<sup>6</sup>, S. Siira<sup>6</sup>, A. Filipovska<sup>6</sup>, A. Munnich<sup>1,4</sup>, C. F. Patrick<sup>2</sup>, R. McFarland<sup>2</sup>, A. Rötig<sup>1</sup>, R. W. Taylor<sup>2</sup>;*

<sup>1</sup>INSERM U1163, Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Paris, France, <sup>2</sup>Wellcome Trust Centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, United Kingdom, <sup>3</sup>Willink Biochemical Genetics Unit, Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, <sup>4</sup>Departments of Pediatric, Neurology and Genetics, Hôpital Necker-Enfants-Malades, Paris, France, <sup>5</sup>Wellcome Trust Centre for Mitochondrial Research, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom, <sup>6</sup>Harry Perkins Institute of Medical Research, Centre for Medical Research and School of Chemistry and Biochemistry, The University of Western Australia, Perth, Australia.

Mitochondrial disorders are both clinically and genetically diverse since defective expression of mitochondrial (mt)DNA can be caused by mitochondrial or nuclear gene mutations. Recently, several novel genes encoding factors involved in mt-tRNA processing have been identified as important causes of mitochondrial disease. Using whole exome sequencing, we identified mutations in the *TRMT10C* gene (encoding the mitochondrial protein MRPP1), in two unrelated individuals who presented at birth with elevated CSF and serum lactate levels, hypotonia and deafness. Together, MRPP1, MRPP2 and MRPP3 form the mitochondrial ribonuclease P (mt-RNase P) protein that is responsible for cleaving the 5' end of mt-tRNAs from polycistronic precursor RNA molecules. Additionally, a stable complex of MRPP1 and MRPP2 has m<sup>1</sup>R9 methyltransferase activity which methylates mt-tRNAs at position 9, a vital modification required for folding mt-tRNAs into their correct tertiary structures. Analysis of subject fibroblasts harboring *TRMT10C* missense variants revealed decreased protein levels of MRPP1 and defective mitochondrial protein synthesis. Cell lines from affected individuals showed an increase in mt-tRNA precursors indicative of defective mt-tRNA processing. The pathogenicity of the detected variants - compound heterozygous p.(Arg181Leu) and p.(Thr272Ala) changes in subject 1, a homozygous p.(Arg181Leu) variant in subject 2 - was confirmed by the functional rescue of associated OXPHOS and mt-tRNA processing defects following lentiviral transduction of wild type *TRMT10C*. Our study suggests that these variants affect MRPP1 stability and mt-tRNA processing without effects on m<sup>1</sup>R9 methyltransferase activity, identifying *TRMT10C* as a novel mitochondrial disease gene and highlighting the importance of post-transcriptional RNA processing for mitochondrial function.

## C17.3

**Mitochondrial inorganic pyrophosphatase (PPA2) mutations underlie a spectrum of cardiomyopathy disorders**

*K. Doudney<sup>1</sup>, H. Kennedy<sup>1</sup>, T. B. Haack<sup>2</sup>, V. Hartill<sup>3</sup>, L. Matakovic<sup>4</sup>, E. Baumgartner<sup>5</sup>, H. Potter<sup>1</sup>, R. Mackay<sup>1</sup>, C. Alston<sup>1</sup>, S. O'Sullivan<sup>1</sup>, R. McFarland<sup>1</sup>, G. Connolly<sup>6</sup>, C. Gannon<sup>7</sup>, R. King<sup>1</sup>, S. Mead<sup>1,10</sup>, I. Crozier<sup>11</sup>, W. Chan<sup>11</sup>, C. Florkowski<sup>1</sup>, M. Sage<sup>12</sup>, T. Höfken<sup>13</sup>, B. Alhaddad<sup>14</sup>, L. Kremer<sup>2</sup>, R. Kopajtich<sup>2</sup>, R. Feichtinger<sup>15</sup>, W. Sperli<sup>15</sup>, R. Rodenburg<sup>16</sup>, J. Minet<sup>17</sup>, A. Dobbie<sup>18</sup>, T. Strom<sup>2</sup>, T. Meitinger<sup>2</sup>, P. George<sup>1</sup>, C. Johnson<sup>19</sup>, R. Taylor<sup>6</sup>, H. Prokisch<sup>2</sup>, J. Mayr<sup>15</sup>;*

<sup>1</sup>Molecular Pathology Laboratory, Canterbury Health Laboratories, Canterbury District Health Board, Christchurch, New Zealand, <sup>2</sup>Institute of Human Genetics, Technische Universität München, Munich, Germany, <sup>3</sup>University of Leeds, St James' University Hospital, Leeds, United Kingdom, <sup>4</sup>Paracelsus Medical University Salzburg, Salzburg, Austria, <sup>5</sup>Metabolic Unit, University Children's Hospital Basel, Basel, Switzerland, <sup>6</sup>Wellcome Trust Centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University, Newcastle, United Kingdom, <sup>7</sup>Royal Hospital for Sick Children, Belfast, United Kingdom, <sup>8</sup>Department of Clinical Biochemistry, Royal Victoria Hospital, Belfast, United Kingdom, <sup>9</sup>Department of Pathology, Royal Victoria Hospital, Belfast, United Kingdom, <sup>10</sup>Genetics Laboratory, Prince of Wales Hospital, Sydney, Australia, <sup>11</sup>Department of Cardiology, Christchurch Hospital, Canterbury District Health Board, Christchurch, New Zealand, <sup>12</sup>Department of Anatomical Pathology, Christchurch Hospital, Canterbury District Health Board, Christchurch, New Zealand, <sup>13</sup>Department of Life Sciences, Brunel University London, London, United Kingdom, <sup>14</sup>Institute of Human Genetics, Technische Universität München, München, Germany, <sup>15</sup>Department of Pediatrics, Paracelsus Medical University Salzburg, Salzburg, Austria, <sup>16</sup>Department of Pediatrics, Nijmegen Center for Mitochondrial Disorders, Radboud University Medical Centre, Nijmegen, Netherlands, <sup>17</sup>Department of Neonatology UKBB Bruderholz, University Children's Hospital Basel, Basel, Switzerland, <sup>18</sup>Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds, United Kingdom, <sup>19</sup>Section of Ophthalmology & Neurosciences, Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Leeds, United Kingdom.

We have identified biallelic missense mutations in the nuclear-encoded mitochondrial inorganic pyrophosphatase (PPA2) in a family with four affected individuals, two of whom died separately in their second decade following the intake of small amounts of alcohol. Post-mortem results identified mid-myocardial fibrosis, however years of biochemical and genetic investigation failed to elucidate possible causes and any links to their highly sensitive alcohol response. Whole exome sequencing of the family revealed

compounding mutations in PPA2, a nuclear encoded mitochondrial protein most commonly associated with complex V in the oxidative phosphorylation respiratory chain. A further three unrelated pedigrees with six acutely affected neonatal individuals were recently identified with homozygous and compound heterozygous PPA2 mutations, resulting in much more severe symptoms including seizures, lactic acidosis and cardiac arrhythmia leading to death within days or months of birth. Comparison of fibroblast mitochondria from normal and mutated PPA2 individuals showed the activity of inorganic pyrophosphatase was significantly reduced in affected individuals. Recombinant PPA2 enzymes modelling hypomorphic missense mutations exhibit decreased activity that correlated with disease severity. These findings confirm the pathogenicity of PPA2 mutations, and suggest that PPA2 is a new cardiomyopathy-associated protein, which has a greater physiological importance in mitochondrial function than previously recognised.

## C17.4

**Biallelic mutations of PPA2 (pyrophosphatase inorganic 2) in 2 families with recurrence of sudden unexpected infant death**

*A. Guimier<sup>1</sup>, C. Gordon<sup>1</sup>, F. Godard<sup>2</sup>, M. Oufadem<sup>1</sup>, C. Vasnier<sup>3</sup>, P. Nitschke<sup>1</sup>, C. Boile-Feyot<sup>4</sup>, C. Masson<sup>1</sup>, S. Dauger<sup>4</sup>, C. Rambaud<sup>5</sup>, B. Kugener<sup>6</sup>, D. Bonnet<sup>7</sup>, P. Bouvagnet<sup>8</sup>, S. Di Filippo<sup>9</sup>, V. Probst<sup>10</sup>, R. Redon<sup>10</sup>, P. Charron<sup>11</sup>, A. Rötig<sup>1</sup>, S. Lyonnet<sup>1</sup>, L. De Pontual<sup>1</sup>, J. di Rago<sup>2</sup>, A. Delahodde<sup>3</sup>, J. Amiel<sup>1</sup>;*

<sup>1</sup>Institut Imagine, INSERM U1163, Paris, France, <sup>2</sup>Univ. Bordeaux, CNRS, Institute of Cellular Biochemistry and Genetics (IBGC, UMR 5095), Bordeaux, France, <sup>3</sup>Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France, <sup>4</sup>Pediatric Intensive Care Unit, Robert Debré University Hospital, AP-HP, Paris, France, <sup>5</sup>Forensics and Pathology Department, Raymond Poincaré University Hospital, AP-HP, Garches, France, <sup>6</sup>CRRMIN Rhône Alpes, Bron, France, <sup>7</sup>Unité Médico-Chirurgicale de Cardiologie Congénitale et Pédiatrique, Centre de Référence Malformations Cardiaques Congénitales Complexes (M3C), Hôpital Necker-Enfants Malades, AP-HP, Paris, France, <sup>8</sup>Laboratoire Cardiogénétique, Hôpices Civils de Lyon, Lyon, France, <sup>9</sup>Service de cardiologie pédiatrique, Hôpices Civils de Lyon, Lyon, France, <sup>10</sup>L'institut du thorax, Inserm UMR1087 CNRS UMR6291, Nantes, France, <sup>11</sup>Départements de génétique et de cardiologie, Centre de référence des maladies cardiaques héréditaires, Hôpital Pitié-Salpêtrière, Paris, France.

Sudden unexpected death in infancy (SUDI) occurs in apparently healthy infants and remains unexplained despite thorough investigation including complete autopsy. The vast majority of cases are sporadic. We investigated two families with recurrence of SUDI in 4/5 and 2/2 siblings between 4 and 20 months of age. Whole exome sequencing of two affected cases in family 1 and one in family 2 revealed compound heterozygous missense variations in the *PPA2* gene, encoding mitochondrial pyrophosphatase inorganic 2. This protein is thought to catalyze hydrolysis of inorganic pyrophosphate, the homeostasis of which is exceedingly important for the cell. The four missense variations were confirmed by Sanger sequencing and segregated according to autosomal recessive inheritance in both families. PPA2 protein levels were decreased in the patients' fibroblasts compared to controls. We show that yeast cells lacking the orthologous gene (*ppa2Δ*) are not viable due to the loss of mitochondria and are rescued by the human *PPA2* gene but not when it carries the mutations found in patients. Using a regulatable (doxycycline-repressible) gene expression system we show that the primary consequences of the pathogenic *ppa2* mutations are a decreased rate of oxygen consumption and ATP synthesis and a drop in the electrical mitochondrial membrane potential. Altogether these data demonstrate that *PPA2* is an essential gene in yeast and that biallelic mutations in *PPA2* cause a novel mitochondrial disease associated with sudden death in infants.

## C17.5

**Mutations in TMEM126B cause a severe isolated complex I deficiency and variable clinical phenotype**

*C. L. Alston<sup>1</sup>, A. G. Compton<sup>2,3</sup>, L. E. Formosa<sup>4</sup>, V. Strecker<sup>5</sup>, M. Oláhová<sup>1</sup>, T. B. Haack<sup>6,7</sup>, J. Smet<sup>8</sup>, K. Stouffs<sup>9</sup>, P. Diakumis<sup>10,11</sup>, E. Ciara<sup>12</sup>, D. Cassiman<sup>13</sup>, N. Romain<sup>14,15</sup>, J. W. Yarham<sup>1</sup>, L. He<sup>1</sup>, B. De Paepe<sup>6</sup>, A. V. Vanlander<sup>8</sup>, S. Seneca<sup>9</sup>, R. G. Feichtinger<sup>16</sup>, R. Ploski<sup>17</sup>, D. Rokicki<sup>18</sup>, E. Pronicka<sup>12,19</sup>, R. G. Haller<sup>15,14</sup>, M. Bahlo<sup>10,11</sup>, J. Mayr<sup>16</sup>, R. Van Coster<sup>8</sup>, H. Prokisch<sup>6,7</sup>, I. Wittig<sup>5,19,20</sup>, M. T. Ryan<sup>4</sup>, D. R. Thorburn<sup>3,2</sup>, R. W. Taylor<sup>1</sup>;*

<sup>1</sup>Wellcome Trust Centre for Mitochondrial Research, Newcastle upon Tyne, United Kingdom, <sup>2</sup>Dept. of Paediatrics, University of Melbourne, Melbourne, Australia, <sup>3</sup>Murdoch Childrens Research Institute & Victorian Clinical Genetic Services, Royal Children's Hospital, Melbourne, Australia, <sup>4</sup>Dept. of Biochemistry & Molecular Biology, Monash University, Melbourne, Australia, <sup>5</sup>Functional Proteomics, SFB 815 Core Unit, Goethe-Universität, Frankfurt, Germany, <sup>6</sup>Inst. of Human Genetics, TU-München, Munich, Germany, <sup>7</sup>Inst. of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany, <sup>8</sup>Dept. of Pediatric Neurology & Metabolism, Ghent University Hospital, Ghent, Belgium, <sup>9</sup>Center for Medical Genetics, UZ Brussel, Research Group Reproduction & Genetics, Vrije Universiteit Brussels, Brussels, Belgium, <sup>10</sup>Bioinformatics Div, Walter and Eliza Hall Inst. of Medical Research, Melbourne, Australia, <sup>11</sup>Dept. of Mathematics and Statistics, University of Melbourne, Melbourne, Australia, <sup>12</sup>Dept. of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland, <sup>13</sup>Metabolic Center, University

Hospitals Leuven, Leuven, Belgium, <sup>14</sup>Dept. of Neurology & Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX, United States, <sup>15</sup>Neuromuscular Center, Inst. for Exercise & Environmental Medicine, Texas Health Presbyterian Hospital, Dallas, TX, United States, <sup>16</sup>Dept. of Pediatrics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria, <sup>17</sup>Dept. of Medical Genetics, Warsaw Medical University, Warsaw, Poland, <sup>18</sup>Dept. of Pediatrics, Nutrition & Metabolic Diseases, The Children's Memorial Health Institute, Warsaw, Poland, <sup>19</sup>Cluster of Excellence "Macromolecular Complexes", Goethe-Universität, Frankfurt, Germany, <sup>20</sup>German Center for Cardiovascular Research, Partner site RheinMain, Frankfurt, Germany.

Isolated complex I deficiency is the most common biochemical phenotype observed in patients with mitochondrial disease, and with an estimated 1300 proteins in the mitoproteome it is often associated with clinical and genetic heterogeneity. Massively parallel sequencing (MPS) technologies including custom, targeted gene panels or unbiased whole exome sequencing (WES) are hugely powerful in identifying the underlying genetic defect in a clinical diagnostic setting, yet a significant proportion of patients still lack a genetic diagnosis. These patients may harbour mutations in poorly understood or uncharacterised genes, and their diagnosis relies upon characterisation of these orphan genes. Complexome profiling has recently identified TMEM126B as a component of the mitochondrial complex I assembly (MCIA) complex alongside the ACAD9, ECSIT, NDUFAF1 and TIMMDC1 proteins. We describe the clinical, biochemical and molecular findings in six cases of TMEM126B-related mitochondrial disease from four unrelated families. We provide functional evidence to support the pathogenicity of these TMEM126B variants and unequivocally establish this gene as a cause of complex I deficiency in association with either a severe multisystem presentation in infancy or pure myopathy in later adulthood. Functional experimentation including lentiviral rescue and complexome profiling of subject cell lines establishes TMEM126B as the tenth complex I assembly factor associated with human disease and validates the importance of proteomic approaches in characterising novel disease genes whose physiological roles were previously undetermined.

## C17.6

### A syndrome characterized by recurrent episodes of acute liver failure, peripheral neuropathy, cerebellar vermis atrophy, and ataxia is caused by disruptive mutations in SCYL1

R. E. Bittner<sup>1</sup>, W. M. Schmidt<sup>1</sup>, B. Mayerhofer<sup>1</sup>, S. Rutledge<sup>2</sup>, R. Schüle<sup>3,4,5</sup>, S. Züchner<sup>5</sup>, E. Boltshauser<sup>6</sup>;

<sup>1</sup>Medical University of Vienna, Vienna, Austria, <sup>2</sup>Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, United States, <sup>3</sup>Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany, <sup>4</sup>German Research Center for Neurodegenerative Diseases, University of Tübingen, Tübingen, Germany, <sup>5</sup>Dr John T Macdonald Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL, United States, <sup>6</sup>Division of Pediatric Neurology, University Children's Hospital, Zurich, Zürich, Switzerland.

Hereditary cerebellar ataxias are heterogeneous disorders characterized by gait disturbances, often accompanied by additional neurological symptoms and involvement of other organs. Here, we report on three human individuals from two unrelated families, who presented with recurrent episodes of acute liver failure in early infancy, peripheral neuropathy, cerebellar vermis atrophy, and ataxia. By whole exome-sequencing, we identified compound heterozygous mutations within the SCYL1 gene in all affected individuals. All mutations identified were predictably disruptive, causing loss of SCYL1 at the protein level. Despite clear parallels in clinical phenotypes between a Scyl1 deficient mouse model and human patients, recurrent hepatic failure represents a non-neurological clinical manifestation that has not been anticipated from the mouse study. SCYL1 belongs to the evolutionarily highly conserved SCY1-like family of catalytically inactive protein kinases and plays an important constituent of the coatomer (COPI)-coated vesicles mediated membrane trafficking machinery. We showed that in SCYL1-deficient human fibroblasts the Golgi apparatus is massively enlarged, substantiating the notion that SCYL1 maintains Golgi morphology by interacting with several key components of COPI coats. Intriguingly, SCYL1 shares a functional role in retrograde Golgi transport with the NBAS gene, which was recently implicated in a syndromic as well as a non-syndromic form of recurrent episodes of infantile liver failure, which suggests a common pathogenetic underpinning.

Our study demonstrates that the discovery of human disease related genes can be accelerated by studying naturally occurring mouse mutants and further argues for an unbiased genome-wide strategy in the molecular diagnosis of patients with rare inherited disorders.

## C18 Eye Disorders

### C18.1

#### PTCH1 is a major contributor to ocular developmental anomalies

P. Calvas<sup>1</sup>, E. E. Davis<sup>2</sup>, A. Causse<sup>3</sup>, V. David<sup>4</sup>, A. Demaison<sup>1</sup>, A. R. Niederriter<sup>5</sup>, S. Lamarre<sup>6</sup>, C. Vincent-Delorme<sup>7</sup>, L. Pasquier<sup>8</sup>, C. Coubes<sup>9</sup>, D. Lacombe<sup>10</sup>, M. Rossi<sup>11</sup>, J. Dufier<sup>12</sup>, H. Dollfus<sup>13</sup>, J. Kaplan<sup>14</sup>, N. Katsanis<sup>15</sup>, H. C. Etchevers<sup>16</sup>, S. Faguer<sup>17</sup>, N. Chassaing<sup>1</sup>;

<sup>1</sup>UMR1056 INSERM/UPS, Toulouse, France, <sup>2</sup>Center for Human Disease Modeling, Duke University Medical Center, Durham, NC, United States, <sup>3</sup>Ophthalmology department, Toulouse, France, <sup>4</sup>Institut de Génétique et Développement, CNRS UMR6290, Université de Rennes 1, IFR140 GPAS, Rennes, France, <sup>5</sup>Center for Human Disease Modeling, Duke University Medical Center, Durham, NC, United States, <sup>6</sup>INRA, UMR792, & Plateforme Biopuces de la Génopole de Toulouse Midi Pyrénées, INSA/ DGBA 135, Toulouse, France, <sup>7</sup>Service de Génétique Médicale, Hôpital Jeanne de Flandre, Lille, France, <sup>8</sup>Service de Génétique clinique, Hôpital Sud, Rennes, France, <sup>9</sup>Service de Génétique Médicale, Hôpital Arnaud de Villeneuve, Montpellier, France, <sup>10</sup>Service de Génétique Médicale, Hôpital Pellegrin, Bordeaux, France, <sup>11</sup>Service de Génétique, Hospices Civils de Lyon, INSERM U1028 UMR CNRS 5292, UCBL, CRNL TIGER Team, Lyon, France, <sup>12</sup>Service d'Ophtalmologie, Hôpital Necker enfants Malades, Paris, France, <sup>13</sup>Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, <sup>14</sup>UMR1056 INSERM Université ParisV, LGO Institut Imagine des maladies génétiques, Paris, France, <sup>15</sup>Center for Human Disease Modeling, Department of Pediatrics and Department of Cell Biology, Duke University Medical Center, Durham, NC, United States, <sup>16</sup>UMR910 INSERM, Université de la Méditerranée Faculté de Médecine, Marseille, France, <sup>17</sup>UMR1048 INSERM/UPS, I2MC, Toulouse, France.

Ocular developmental anomalies (ODA) including Anophthalmia/Microphthalmia (AM) have an estimated combined prevalence of 3.7 in 10,000 births.

Mutations in SOX2 are the most frequent contributors to severe ODA, yet account for a minority of the genetic drivers. To identify novel ODA loci, we conducted targeted high-throughput sequencing of 407 candidate genes in an initial cohort of 22 sporadic ODA patients. Patched 1 (PTCH1), an inhibitor of sonic hedgehog (SHH) signaling, harbored an enrichment of rare heterozygous variants in comparison to either controls, or to the other candidate genes; targeted resequencing of PTCH1 in a second cohort of 48 ODA patients identified two additional rare nonsynonymous changes. Using multiple transient models and a CRISPR/Cas9 mutant, we show physiologically relevant phenotypes altering SHH signaling and eye development upon abrogation of *ptch1* in zebrafish for which in vivo complementation assays using these models showed that all six patient missense mutations affect SHH signaling. Finally, through transcriptomic and ChIP analyses, we show that SOX2 binds to an intronic domain of the PTCH1 locus to regulate PTCH1 expression, findings that were validated both in vitro and in vivo.

These results demonstrate that PTCH1 mutations contribute to as much as 10% of ODA, identify the SHH signaling pathway as a novel effector of SOX2 activity during human ocular development, and indicate that ODA is likely the result of overactive SHH signaling in humans harboring mutations in either PTCH1 or SOX2.

### C18.2

#### Non-coding mutations in the promoter of OVOL2 cause autosomal dominant corneal endothelial dystrophies congenital hereditary endothelial dystrophy 1 (CHED1) and posterior polymorphous corneal dystrophy 1 (PPCD1)

A. E. Davidson<sup>1</sup>, P. Liskova<sup>2</sup>, C. J. Evans<sup>1</sup>, L. Dudakova<sup>2</sup>, L. Nosková<sup>2</sup>, N. Pontikos<sup>3</sup>, C. Zarouchlioti<sup>4</sup>, V. Plagnol<sup>3</sup>, M. Filipec<sup>4</sup>, S. Knoch<sup>5</sup>, S. J. Tuft<sup>5,6</sup>, A. J. Hardcastle<sup>1</sup>;

<sup>1</sup>UCL Institute of Ophthalmology, London, United Kingdom, <sup>2</sup>Charles University in Prague and General Teaching Hospital in Prague, Prague, Czech Republic, <sup>3</sup>UCL Genetics Institute, London, United Kingdom, <sup>4</sup>European Eye Clinic Lexum, Prague, Czech Republic, <sup>5</sup>Moorfields Eye Hospital, London, United Kingdom.

Congenital hereditary endothelial dystrophy 1 (CHED1) and posterior polymorphous corneal dystrophy 1 (PPCD1) are autosomal dominant corneal endothelial dystrophies which map to overlapping loci on chromosome 20. To identify the genetic cause(s) of disease we recruited extensive pedigrees comprising over 100 affected individuals. Whole-genome sequencing and targeted re-sequencing experiments resulted in identification of unique heterozygous mutations within a conserved region of the OVOL2 proximal promoter sequence; c.-339\_361dup in a large British CHED1 pedigree and c.-370T>C in a Czech PPCD1 cohort. In additional families, we identified two other mutations within the highly conserved proximal promoter sequence (c.-274T>G and c.-307T>C) by direct sequencing of the OVOL2 promoter. OVOL2 is a transcription factor and a direct transcriptional repressor of the established PPCD-associated gene ZEB1. Both transcription factors regulate mesenchymal-to-epithelial transition. Using a dual luciferase reporter assay we demonstrated that all four OVOL2 promoter mutations display significantly ( $P \leq 0.001$ ) increased transcriptional activity compared to the

corresponding wild-type promoter sequence. Furthermore we demonstrate that OVOL2 is not normally expressed in healthy adult corneal endothelium and in vitro culture of patient derived primary corneal endothelial cells (CECs) harbouring an OVOL2 promoter mutation reveals aberrant OVOL2 expression. Our data establish CHED1 and PPCD1 as allelic conditions and implicates transcriptional dysregulation of OVOL2 as a common cause of dominantly inherited corneal endothelial dystrophies. We hypothesise that the non-coding mutations identified create cryptic cis-acting regulatory sequence binding sites that drive the aberrant OVOL2 expression detected in patient derived CECs.

### C18.3

#### Gillespie Syndrome: a unique gene, two modes of inheritance

L. Fares-Taie<sup>1</sup>, S. Gerber<sup>1</sup>, K. J. Alzayady<sup>2</sup>, L. Burglen<sup>3</sup>, V. Marchesin<sup>4</sup>, O. Roche<sup>5</sup>, M. Rio<sup>6</sup>, B. Funalot<sup>7</sup>, R. Calmon<sup>8</sup>, A. Durr<sup>9</sup>, V. Gil da Silva Lopes<sup>10</sup>, M. Ribeiro Bittar<sup>10</sup>, B. Heron<sup>11</sup>, P. Berquin<sup>12</sup>, N. Bahi Buisson<sup>13</sup>, C. Bole<sup>14</sup>, C. Masson<sup>15</sup>, A. Munnich<sup>6</sup>, D. Bremond Gignac<sup>5</sup>, M. Simons<sup>4</sup>, N. Boddaert<sup>16</sup>, S. Lyonnet<sup>6</sup>, J. Kaplan<sup>1</sup>, P. Calvas<sup>17</sup>, D. Yule<sup>2</sup>, J. Rozet<sup>1</sup>;

<sup>1</sup>Laboratory of Genetics Ophthalmology Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>2</sup>Department of Pharmacology and physiology, University of Rochester, New York, NY, United States, <sup>3</sup>Reference center for cerebellar malformations and congenital diseases INSERM U1141 DHU PROTECT Robert Debre Hospital, Paris, France, <sup>4</sup>Epithelial biology and disease Liliane Bettencourt Chair of Developmental Biology INSERM U1163 Imagine Institut Paris Descartes University, Paris, France, <sup>5</sup>Department of Ophthalmology IHU Necker University Paris Descartes, Paris, France, <sup>6</sup>Department of Genetics IHU Necker University Paris Descartes, Paris, France, <sup>7</sup>Department of Genetics GHU Henri Mondor, Créteil, France, <sup>8</sup>Department of Neuroradiology IHU Necker Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>9</sup>Institut du cerveau et de la moelle épinière CHU Paris GH La Pitié Salpêtrière Charles Foix, Paris, France, <sup>10</sup>Departamento de Genética Médica Faculdade de Ciencias UNICAMP, Campinas, Brazil, <sup>11</sup>Department of Neuropediatrics Hopital Trousseau, Paris, France, <sup>12</sup>Pôle de pédiatrie CHU Amiens Hopital Nord, Amiens, France, <sup>13</sup>Embryology and genetics of human malformation Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>14</sup>Genomics Platform Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>15</sup>Bioinformatics Platform Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>16</sup>Department of Neuroradiology IHU Necker Imagine Institut INSERM U1163 Paris Descartes University, Paris, France, <sup>17</sup>Service de Génétique Hopital Purpan, Toulouse, France.

**Introduction.** Gillespie syndrome (GS) is a rare variant form of aniridia characterized by nonprogressive cerebellar ataxia, intellectual disability, and iris hypoplasia. Unlike the more common dominant and sporadic forms of aniridia, there has been no significant association with PAX6 mutations in individuals with GS and the mode of inheritance of the disease had long been regarded as uncertain. The aim of the present study was to characterize the molecular bases of GS.

**Materiel and Methods.** We used a combination of trio-based whole exome sequencing and Sanger sequencing in five simplex GS families. Functional analysis of the identified mutations was assessed in cell models.

**Results and conclusions.** We found homozygous or compound heterozygous truncating mutations and de novo heterozygous mutations in a unique gene supporting the genetic homogeneity of this rare disease. The gene encodes a protein involved in calcium homeostasis. Expression in a heterologous cell system of the truncation mutants and of the missense mutants -alone or in combination with the wildtype counterpart- supported that both loss-of-function and dominant negative mutations cause Gillespie syndrome ending the long debate regarding the transmission of this rare syndrome.

### C18.4

#### Hypomorphic mutations in RCBTB1 cause autosomal recessive isolated and syndromic inherited retinal dystrophy

F. Coppieters<sup>1</sup>, G. Ascardi<sup>2</sup>, K. Nikopoulos<sup>3</sup>, M. Xu<sup>4</sup>, K. Dannhausen<sup>4</sup>, M. K. Tsilimbaris<sup>5</sup>, T. Chrysanthi<sup>5</sup>, B. Stylian<sup>6</sup>, M. Karlstetter<sup>4</sup>, M. Bauwens<sup>1</sup>, M. De Bruyne<sup>1</sup>, F. Meire<sup>6</sup>, T. Langmann<sup>6</sup>, R. Chen<sup>3</sup>, C. Rivolta<sup>2</sup>, C. P. Hamel<sup>7</sup>, B. P. Leroy<sup>8,9</sup>, E. De Baere<sup>1</sup>;

<sup>1</sup>Center for Medical Genetics, Ghent University Hospital, Ghent, Ghent, Belgium,

<sup>2</sup>Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland,

<sup>3</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States <sup>4</sup>Department of Ophthalmology, University of Cologne, Cologne, Germany, <sup>5</sup>Department of Ophthalmology, Medical School, University of Crete, Heraklion, Greece, <sup>6</sup>Department of Ophthalmology, Hôpital des Enfants Reine Fabiola, Brussels, Belgium, <sup>7</sup>INSERM U 1051, Institut des Neurosciences de Montpellier, Montpellier, France, <sup>8</sup>Department of Ophthalmology, Ghent University Hospital and Ghent University, Ghent, Belgium, <sup>9</sup>Division of Ophthalmology, The Children's Hospital of Philadelphia, Philadelphia, PA, United States.

We identified a homozygous missense variant in RCBTB1 c.973C>T p.(His325Tyr) in individuals with a syndromic inherited retinal dystrophy (IRD), consisting of retinitis pigmentosa (RP), hypothyroidism, primary ovarian insufficiency, and mild intellectual disability. Here, we aimed to investigate the contribution of RCBTB1 in IRD and to assess the underlying pathogenetic mechanism.

Whole exome sequencing data of over 1000 IRD patients were inspected for RCBTB1 mutations, and targeted next-generation sequencing of the coding region of RCBTB1 was performed in 280 IRD patients. In six families with isolated and syndromic IRD five distinct homozygous missense variants were found. All changes segregate with disease, affect highly conserved amino acids and are predicted to be deleterious. A Mediterranean founder haplotype was identified for mutation c.919G>A, p.(Val307Met), occurring in two families of Italian and Greek origin, respectively. Ocular phenotypes range from typical RP starting in the second decade to chorioretinal dystrophy with a later age of onset. RCBTB1 mRNA expression was demonstrated in human retina and RPE, and protein immunostaining was observed mainly in the inner retina. As RCBTB1 has previously been identified as a Cullin3 substrate adaptor, different components of the Cullin3 and Nrf2 pathway were quantified. This revealed a decreased mRNA expression of Nrf2 and five Nrf2 target genes.

In conclusion, hypomorphic RCBTB1 missense mutations were identified in families with non-syndromic and syndromic IRD respectively, putting forward RCBTB1 as a new IRD disease gene. Finally, our data suggest a potential role of the ubiquitination pathway in the pathogenetic mechanism underlying RCBTB1-associated IRD.

### C18.5

#### Novel genes and phenotypic correlations in inherited retinal disease

G. Arno<sup>1,2</sup>, A. Fiorentino<sup>1,2</sup>, N. Pontikos<sup>1,3</sup>, C. Chakarova<sup>4</sup>, A. G. Robson<sup>1,2</sup>, UKIRDC, G. E.

Holder<sup>1,2</sup>, M. E. Cheetham<sup>1</sup>, V. Plagnol<sup>1,2</sup>, M. Michaelides<sup>1,2</sup>, A. J. Hardcastle<sup>1</sup>, A. R. Webster<sup>1,2</sup>,

<sup>1</sup>UCL Institute of Ophthalmology, London, United Kingdom, <sup>2</sup>Moorfields Eye Hospital, London, United Kingdom, <sup>3</sup>UCL Genetics Institute, London, United Kingdom.

**Introduction:** Inherited retinal disease displays vast genetic and allelic heterogeneity. On cohorts from a UK-wide consortium we used an integrated genomic and phenotypic approach to i) accelerate novel gene discovery ii) determine the phenotypic and severity spectrum for specific molecular subtypes and iii) accumulate whole exome and genome data to identify alleles modifying the action of known genes.

**Methods:** Extensive pre-screening using next-generation sequencing of known genes on over 1000 inherited retinal disease families led to a cohort likely enriched for novel genes for further whole exome/genome sequencing. An integrated database-server was developed based on the ExAC and Phenotips software to query genes and phenotypes (using human phenotype ontology (HPO)) in the pool of families.

**Results:** To date, 84 families have undergone analysis (76 exome, 8 genome). In 13 probands, likely disease causing mutations in known genes were revealed. Amongst these, specific alleles of ceroid lipofuscinosis 7 (*CLN7/MFSD8*) were consistently associated with a non-syndromic retinal degeneration. Three genes, previously unreported in human retinal disease, have been discovered. These include *GNB3* mutated homozygously (c.124C>T ; p.Arg42Ter) in a patient with a distinct stationary retinal dysfunction. 102 exomes of probands with inherited retinal dystrophy (from UCLEx) with detailed phenotypic data have been integrated into a computer searchable framework.

**Discussion:** Pooling, sharing and integration of genomic and phenotypic data increases the power of gene discovery. An extensible, programmatic infrastructure which includes both genomic and phenotypic data has been developed to efficiently determine genotype-phenotype correlations and modifier alleles.

**Support:** RPFB, NIHR-BRC, Fight For Sight.

### C18.6

#### Molecular inversion probe based sequence analysis of 108 genes associated with non-syndromic inherited retinal disease in 4,000 probands

M. Imran Khan<sup>1</sup>, C. Gilissen<sup>1</sup>, C. Ayuso<sup>2</sup>, D. Sharon<sup>3</sup>, R. K. Koenekoop<sup>4</sup>, C. Rivolta<sup>5</sup>, E. de Baere<sup>6</sup>, C. F. Inglehearn<sup>7</sup>, M. van de Vorst<sup>8</sup>, S. Kohl<sup>9</sup>, C. P. Hamel<sup>10</sup>, A. Hoischen<sup>1,10</sup>, F. P. M. Cremers<sup>1,10</sup>,

<sup>1</sup>Department of Human Genetics, Radboud university medical centre, Nijmegen, Netherlands, <sup>2</sup>Department of Genetics, University Hospital Fundación Jiménez Díaz, Madrid, Spain, <sup>3</sup>Departments of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel, <sup>4</sup>Departments of Paediatric Surgery, Human Genetics and Ophthalmology, McGill University Health Center, Montreal, QC, Canada, <sup>5</sup>Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland, <sup>6</sup>Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, <sup>7</sup>Department of Ophthalmology and Neuroscience, University of Leeds, Leeds, United Kingdom, <sup>8</sup>Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany, <sup>9</sup>National Center in Genetic of Sensory Diseases, Institute for Neurosciences of Montpellier, University of Montpellier, Montpellier, France, <sup>10</sup>Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands.

**Purpose:** Inherited retinal diseases (IRDs) are clinically and genetically very heterogeneous, as ~125 genes have been associated with non-syndromic IRDs. The purpose of this study was to develop a flexible, comprehensive and cost-effective sequencing procedure for IRDs.

**Methods:** About 4,000 probands with non-syndromic IRDs were ascertained by partners of the European Retinal Disease Consortium (ERDC). In total, 6,200 molecular inversion probes (MIPs) were designed to capture ~1,600 protein-coding exons and flanking intronic sequences of 108 IRD-associated genes. The captured targets were sequenced on a NextSeq500 Illumina sequencer and the data were analyzed using an in-house pipeline to find the causal genetic variants.

**Results:** In pools of 120 samples the average coverage per probe was ~500X, where 95% of the probes were covered >10X. Analysis was completed for 2,500/4,000 samples. Sanger validation was performed for a representative set of variants identified in 290 probands from Nijmegen. We have identified the causal variants in 59% (172/290) of the cases, which included a number of plausible de novo variants and several novel genotype-phenotype correlations. Material costs for sequencing 108 IRD genes was €65 per sample, rendering it very cost-effective.

**Conclusions:** Taken into consideration that the Nijmegen cohort was previously prescreened using various genotyping methods, the corrected yield would be ~71%. At 1/10th of WES cost, this efficiency is equal or superior to other published gene-panel (36 - 62%) or WES-based (49 - 66%) sequence analysis. Based on preliminary results, we estimate to identify causal variants in at least 2,000 IRD probands.

## C19 Big data Analyses of Intellectual Disability

### C19.1

#### The prevalence and architecture of dominant developmental disorders

**M. E. Hurles**, Deciphering Developmental Disorders Study; Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Children with severe, undiagnosed developmental disorders (DDs) are enriched for damaging de novo mutations in developmentally important genes. We exome sequenced 4,295 families with children with DDs, and meta-analysed these data with published data on children with similar disorders. We identified over 90 genes in which damaging de novo mutations show genome-wide significant ( $p < 1e-6$ ) evidence for causing developmental disorders, including 15 novel genes reaching this level for the first time. We also identified a novel seizure disorder associated with truncating mutations in SMC1A, a gene in which missense mutations are known to cause a different disorder. We estimated that we have statistical power to detect ~50% of all haploinsufficient genes, and that most haploinsufficient genes causing DDs have already been discovered. Our large number of genome-wide significant findings allow us to compare empirically the power to detect novel DD-associated genes using exome or genome sequencing. We find that, at current cost differentials, exome sequencing has much greater power for novel gene discovery for genetically heterogeneous disorders. Finally, we estimate that ~45% of our cohort likely carry pathogenic de novo mutations in coding sequences, with approximately half operating by a loss-of-function mechanism, and the remainder being gain-of-function or dominant negative in action. By extrapolating from the DDD cohort to the general population, we estimate that de novo dominant developmental disorders have an average birth prevalence of 1 in ~400.

### C19.2

#### Meta-analysis of 2,104 trios provides support for 10 novel candidate genes for intellectual disability

**S. H. Lelieveld<sup>1</sup>, M. R. F. Reijnders<sup>2</sup>, R. Pfundt<sup>2</sup>, H. G. Yntema<sup>2</sup>, E. Kamsteeg<sup>2</sup>, P. de Vries<sup>2</sup>, B. B. A. de Vries<sup>2</sup>, M. H. Willemse<sup>2</sup>, T. Kleefstra<sup>2</sup>, K. Löhner<sup>3</sup>, M. Vreeburg<sup>4</sup>, S. Stevens<sup>4</sup>, I. van der Burgt<sup>2</sup>, E. M. H. F. Bongers<sup>2</sup>, A. P. A. Stegmann<sup>1</sup>, P. Rump<sup>3</sup>, T. Rinne<sup>2</sup>, M. R. Nelen<sup>2</sup>, J. A. Veltman<sup>2,4</sup>, L. E. L. M. Vissers<sup>2</sup>, H. G. Brunner<sup>2,4</sup>, C. Gilissen<sup>2</sup>**;

<sup>1</sup>Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>Department of Human Genetics, Donders Centre for Neuroscience, Radboud University Medical Center, Nijmegen, Netherlands, <sup>3</sup>Department of Genetics, University Medical Center Groningen, Groningen, Netherlands, <sup>4</sup>Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands.

**Introduction:** Intellectual disability (ID) and other neurodevelopmental disorders are in part due to de novo mutations. Large-scale whole-exome sequencing (WES) studies of patient-parent trios have efficiently identified genes enriched for de novo mutations in ID patient cohorts. As part of routine

genetic diagnostics at the Radboud University Medical Center, we sequenced the exome of 820 ID-patients and their parents. Here we utilize this dataset to identify novel genetic causes of ID.

**Methods:** Based on gene specific mutation rates we performed a statistical analysis to detect genes that are significantly enriched for loss-of-function or functional de novo mutations. To achieve the best possible power to identify novel candidate ID genes, we added data from previously published exome-sequencing trio studies giving rise to a combined cohort of 2,104 trios. **Results:** In our cohort of 820 ID patients we identified 4 novel genes significantly enriched for functional or loss-of-function de novo mutations. We reproduce these results in the combined ID-set of 2,104 trios and identify 6 further novel genes enriched for de novo mutations. In addition, we show that similar to known ID genes, these 10 candidate genes are highly intolerant to normal variation. A phenotype comparison of patients with de novo mutations in the same gene provides further support for a shared genetic cause.

**Conclusions:** In summary, we identified 10 novel candidate ID genes by performing a meta-analysis on WES data of 2,104 ID trios, highlighting the potential of unbiased statistical analyses of large trio-based sequencing studies to identify novel candidate genes.

### C19.3

#### Whole exome sequencing in 150 consanguineous families with intellectual disability: high diagnostic yield and identification of novel candidate genes

**M. Reuter<sup>1</sup>, H. Tawamie<sup>1</sup>, R. Buchert<sup>1</sup>, T. Froukh<sup>2</sup>, O. Hosny Gebril<sup>3</sup>, T. Strom<sup>4</sup>, S. Hoffjan<sup>5</sup>, D. Wieczorek<sup>6</sup>, H. Sticht<sup>1</sup>, A. Reis<sup>1</sup>, R. Abou Jamra<sup>1,7</sup>**;

<sup>1</sup>Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany, <sup>2</sup>Philadelphia University, Amman, Jordan, <sup>3</sup>National Research Center, Doky, Cairo, Egypt, <sup>4</sup>Helmholtz Zentrum München, Neuherberg, Germany, <sup>5</sup>University of Bochum, Bochum, Germany, <sup>6</sup>Heinrich-Heine-Universität, Düsseldorf, Germany, <sup>7</sup>University Medical Center Leipzig, Leipzig, Germany.

Autosomal recessive intellectual disability (ARID) represent a significant fraction of ID, especially in consanguineous families. Several hundred ARID genes were identified to date, and many more are presumed. We performed genetic mapping and whole exome sequencing (WES) in 150 consanguineous families with one (29%) or multiple (71%) affected children with ID. Most affected persons showed additional symptoms including muscular hypotonia, epilepsy, and microcephaly. We identified relevant variants in 114 (76%) of families. Among these, 53 are meanwhile confirmed genes and likely clarify the cause of ARID in the corresponding families while in 60 the homozygous variant is located in a candidate gene. Despite the high heterogeneity we found variants in two unrelated families for four already described genes, *AHI1*, *GPR56*, *PRRT2*, and *PLA2G6*. At least one patient has two distinct phenotypes because of two homozygous variants in different genes. In order to prove the pathogenicity of non-truncating variants, we applied a wide spectrum of functional experiments. Until now, we have shown a deleterious effect for 12 variants in 10 genes (*FAR1*, *PGAP1*, *PGAP2*, *EZR*, *EDC3*, *PTEN*, *TAF13*, *FRRS1L*, *ZIP8*, *KIAA0586*). In order to prove the causality of the candidate variants, identifying further likely pathogenic variants in similarly affected individuals is necessary. Therefore we initiated a Consortium of Autosomal Recessive Intellectual Disability (CARID) to share data which allowed identification of additional independent cases for 12 candidate genes, so far. WES in consanguineous families with ID has the highest diagnostic yield and should therefore become part of a first line diagnostic approach.

### C19.4

#### Common morphological and transcriptome changes in Rett spectrum disorders justify a shared therapeutic approach

**A. Pinto<sup>1,2</sup>, E. Landucci<sup>1</sup>, L. Bianciardi<sup>1</sup>, S. Daga<sup>1</sup>, E. Frullanti<sup>1</sup>, M. Brindisi<sup>3</sup>, S. Butini<sup>3</sup>, V. Imperatore<sup>4</sup>, F. Ariani<sup>1,2</sup>, S. Brogi<sup>3</sup>, G. Campiani<sup>3</sup>, A. Renieri<sup>1,2</sup>, I. Meloni<sup>1</sup>**;

<sup>1</sup>Medical Genetics, University of Siena, Siena, Italy, <sup>2</sup>Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy, <sup>3</sup>European Research Centre for Drug Discovery & Development Dept. Biotechnology, Chemistry and Pharmacy University of Siena, Siena, Italy.

Rett syndrome is a neurodevelopmental disorder ranging from the classic *MECP2*-related form to a *FOXP1*-related variant. The *FOXP1*-related variant shows a shorter perinatal normal period and more severe microcephaly. Given the presence of shared signs/symptoms and the overlapping function of the involved genes, we hypothesized a common molecular mechanism behind RTT phenotypic spectrum. Therefore, to better investigate this hypothesis, taking advantage of the breakthrough genetic reprogramming technology, we investigated morphological and RNA-seq transcriptome changes on iPSC-derived neurons from several patients for each disease-related gene. Such analysis led to the identification of a unique neuronal

morphological phenotype probably related to axon guidance signal disruption. RNA-seq analysis showed changes in the expression level of genes related to cell migration, adhesion and to the proper establishment of the GABAergic circuit. The transcriptome analysis also revealed an upregulation of enzymes that regulate the acetylation of tubulin and microtubules-related genes MAP2 and MAPT in the iPSCs-derived neurons for each disease-related gene and a reduction in acetylated  $\alpha$ -tubulin was demonstrated by Western blot. These findings strongly support the involvement of common pathways in the pathomechanism of the whole Rett phenotypic spectrum. Notably, in line with the molecular findings, in vitro experiments with drugs selective for the tubulin acetylation pathway (a newly developed HDAC6 inhibitor) and the GABAergic circuits (repurposed drug) showed a significant reversal of the unique discovered morphological phenotype, providing a real possibility of an efficacious common treatment for Rett disorders.

#### C19.5

#### Haploinsufficiency of MECP2-interacting transcriptional co-repressor SIN3A causes mild intellectual disability by affecting the development of cortical integrity

J. Witteveen<sup>1,2</sup>, M. H. Willemsen<sup>3</sup>, T. C. D. Dombroski<sup>1,2</sup>, N. H. van Bakel<sup>1,2</sup>, W. M. Nillesen<sup>3</sup>, J. A. van Hulzen<sup>1,2</sup>, E. J. R. Jansen<sup>1,2</sup>, I. E. Veenstra-Knol<sup>4</sup>, C. M. A. van Ravenswaaij<sup>4</sup>, J. S. Klein Wassink-Ruiter<sup>4</sup>, P. Rump<sup>4</sup>, M. Vincent<sup>5</sup>, A. David<sup>5</sup>, C. Le Gaignec<sup>5,6</sup>, J. Schieving<sup>7</sup>, C. Gilissen<sup>3</sup>, N. Foulds<sup>8</sup>, T. Strom<sup>9,10</sup>, K. Cremer<sup>11</sup>, A. M. Zink<sup>11</sup>, H. Engels<sup>11</sup>, S. A. de Munnik<sup>3</sup>, J. E. Visser<sup>1</sup>, H. G. Brunner<sup>3,2</sup>, G. J. M. Martens<sup>1,2</sup>, R. Pfundt<sup>3</sup>, S. M. Kolk<sup>1,2</sup>, **T. Kleefstra**<sup>3,2</sup>;

<sup>1</sup>Department of Molecular Animal Physiology, Nijmegen, Netherlands, <sup>2</sup>Donders Institute for Brain, Cognition and Behavior, Nijmegen, Netherlands, <sup>3</sup>Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>4</sup>University Medical Center Groningen, Department of Genetics, Groningen, Netherlands, <sup>5</sup>CHU Nantes, Service de génétique médicale, Nantes, France, <sup>6</sup>INSERM; UMR\_S 957; Laboratoire de Physiopathologie de la Résorption osseuse et Thérapie des tumeurs osseuses primitives, Faculté de Médecine; CHU Hôtel-Dieu, Nantes, France, <sup>7</sup>Department of Neurology, Radboud University Medical Center, Nijmegen, Netherlands, <sup>8</sup>UHS NHS Foundation Trust and Department of Human Genetics and Genomic Medicine, University of Southampton, Southampton, United Kingdom, <sup>9</sup>Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany, <sup>10</sup>Institute of Human Genetics, Technische Universität München, Munich, Germany, <sup>11</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany.

Next-generation sequencing has revealed numerous genes that are associated with a broad range of neurodevelopmental disorders including intellectual disability (ID) and autism spectrum disorders (ASD), but detailed knowledge of their associated protein dysfunction is mostly lacking. Here, we identified dominant mutations in the gene encoding the transcriptional repressor and MeCP2-interactor switch-insensitive 3 family member A (SIN3A) in individuals with predominantly mild ID who display a strikingly similar facial gestalt and various additional features as ASD, microcephaly, short stature, epilepsy, hypermobile joints and hearing loss. Brain MRIs revealed subtle abnormalities, such as corpus callosum hypoplasia and ventriculomegaly. Intriguingly, in vivo functional knockdown of SIN3A using in utero electroporation led to changes in the developing mouse brain such as reduced cortical neurogenesis, altered neuronal identity and aberrant cortico-cortical projections, observations that have a high translational value with the clinical features of our individuals. Together, our data establish that haploinsufficiency of SIN3A is associated with mild ID and associated characteristics as observed in the atypical 15q24 microdeletion syndrome, and that Sin3A is a key transcriptional regulator crucial for proper developmental expansion and connectivity of cortical brain areas.

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#### C19.6

#### Genes controlling cell movement and migration are deregulated in ARID1B-associated ID

G. Vasileiou, M. V. Hadjihannas, A. Reis;  
Institute of Human Genetics, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.

The human BRG1-associated factors (BAF) chromatin remodeling complex (also known as SWI/SNF-A complex) regulates the structure and position of nucleosomes and can facilitate both activation as well as repression of gene transcription. Non-syndromic Intellectual disability (ID) and Coffin-Siris syndrome (CSS) are associated with loss-of-function mutations in various components of the BAF complex, such as ARID1B, which is expressed in the human brain and in mammalian embryonic stem cells. To understand how mutated ARID1B affects human brain development and leads to develop-

mental delay phenotypes, we performed whole transcriptome sequencing (RNA-seq) analysis in fresh peripheral blood lymphocytes from ID individuals harboring ARID1B mutations and control subjects. Pathway analysis of 452 genes showing prominent transcriptional changes between affected and control subjects indicated deregulation in cell movement related processes, especially of embryonic stem cells. In ID individuals, qRT-PCR expression analysis confirmed downregulation of two key regulators of actin organization and inducers of cell migration, Rho-associated protein kinases ROCK1 and ROCK2. Using models for cell migration we found that siRNA-mediated knockdown of ARID1B strongly reduced cell migration and led to downregulation of both ROCK1 and ROCK2. In addition to ROCK1/ROCK2, in both neuroblastoma and osteosarcoma cells, ARID1B knockdown led to downregulation of NCK1, an important mediator of activated receptor tyrosine kinase signaling that reorganizes the actin cytoskeleton and promotes cell polarization and directional migration via pseudopodia formation. Our results indicate that ARID1B can regulate cell migration and raise the possibility that compromised cell migration due to ARID1B mutations could be implicated in ARID1B-associated ID syndromes.

#### C20 Gene Editing: To Fear or to Cheer? (joint with EMPAG)

##### C20.3

##### Are biomedical research fundamental principles appropriate for using genome editing in humans?

E. Rial-Sebbag, A. Cambon-Thomsen;  
INSERM UMR 1027, Toulouse, France.

Genome editing with CRISPR-Cas 9 is considered as a revolution in life sciences since 2013. Thanks to this technology a sequence implicated in a disease could be modified in the DNA of various cell types: somatic, germinal or in embryo. This technology is still under scientific evaluation which poses the question of its validation in embryos and humans and its future applications in therapy. However, the fundamental principles and bioethics basis for conducting research in these three areas can vary according different legal regimes and are implemented into national regulations in a heterogeneous manner.

Beyond these legal regimes Scientific Academies, research institutes and ethics committees have provided materials (reports, opinions) based on core ethical principles coming from the biomedical field for using Genome editing for medical applications. In this paper we analyse the different positions adopted by those bodies and we compare their points of convergence and divergence as well as the ethical and legal principles underlying their conclusions. We then compare the results with the only binding instrument in Europe for Bioethics: the Oviedo Convention (articles 13 and 18). Finally, we survey the fundamental rights principles expected to be relevant for Genome editing: How could they conflict with the research supported by several institutions in Europe? Can an inheritable modification of the genome, illegal according to the Convention, still can be envisaged? EUcelLex project (FP7 grant 601806)

##### C20.4

##### One small edit for man, one large edit for mankind? Points to consider for a responsible way forward with gene editing

H. C. Howard<sup>1</sup>, G. de Wert<sup>2</sup>, C. G. van Eijk<sup>3</sup>, F. Forzano<sup>4</sup>, D. Radovikovic<sup>5</sup>, E. Rial-Sebbag<sup>6</sup>, M. C. Cornel<sup>7</sup>, on behalf of the Public and Professional Policy Committee of the European Society of Human Genetics;

<sup>1</sup>Centre for Research Ethics and Bioethics, Uppsala University, Uppsala, Sweden,

<sup>2</sup>Department of Health, Ethics & Society, Research Schools CAPHRI and GROW, Maastricht University, Maastricht, Netherlands, <sup>3</sup>Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Centre, Amsterdam, Netherlands, <sup>4</sup>Medical Genetics Unit, Ospedali Galliera, Genova, Italy, <sup>5</sup>Laboratory for Molecular Genetics, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>6</sup>INSERM, Faculté de médecine Université Toulouse 3, Paul Sabatier, Toulouse, France, <sup>7</sup>Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

A recent resurgence of debate over genetic modification has been ignited, due to the recent advent of a novel tool for site-specific gene editing, CRISPR-Cas9, and the publication in April 2015 that human embryos had been edited in research. CRISPR-Cas9 allows for increased efficiency, specificity, relative ease and speed of use, and accessibility to researchers. Increased access to, and novel uses of, new technologies leads to the need for further reflection on the potential scientific, and medical impacts as well as ethical,

legal and social issues (ELSI). While some of the risks and ELSI of somatic and heritable gene editing in humans have been discussed previously (e.g. re: IVF, PGD, cloning) what is particular to the current discussion is that we have, technically, never been so close to having the technology to conduct gene editing in humans in a potentially safe and effective manner. Contributing to a constructive discussion within the ESHG, the Public and Professional Policy Committee has outlined different areas that require attention in order to move forward and ensure a responsible use and application of gene editing. These include: education and engagement of different stakeholders, including lay stakeholders to support meaningful discussions; identification and monitoring of risks; ethical reflection identifying generic risks and issues as well as those specific to gene editing; attention to governance and legal issues; addressing the potential roles and impacts of commercial actors in the development of gene editing; and the consideration of alternative methods that could be used instead of gene editing.



## C20.5

### Ethical issues of gene editing: what does popular media report?

**E. Niemiec**<sup>1,2,3</sup>, **B. M. Zimmermann**<sup>4</sup>, **H. C. Howard**<sup>4</sup>;

<sup>1</sup>Joint International PhD Program in Law, Science and Technology, University of Bologna, Bologna, Italy, <sup>2</sup>Department of Law, University of Turin, Turin, Italy, <sup>3</sup>Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Hannover, Germany, <sup>4</sup>Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, <sup>5</sup>Department for Biomedicine, University of Basel, Basel, Switzerland, <sup>6</sup>Centre for Research Ethics and Bioethics, Uppsala University, Uppsala, Sweden.

CRISPR Cas9 gene editing technology was reported to have been used in human embryos for the first time in April 2015. This research led by Junjiu Huang has sparked controversy, prompted debate and extensive media coverage on this issue. Since then, a number of meetings have been held to discuss this issue, and reports, statements, and recommendations have been issued by groups, including the Washington Summit organizers, the Hinxton Group, and The European Group on Ethics in Science and New Technologies. These highlight the need to have an ongoing discussion with a multitude of stakeholders including experts and lay public. Discussing a novel technology with different stakeholders can be difficult if stakeholders are not aware of, or do not have much information about said technology. One of the widely accessible educational as well as opinion-forming resources for lay public about new scientific advancements may be media coverage. To study the information disseminated through the media on gene editing, a content analysis of a subset of English newspaper articles was performed. Articles were identified from five of the most popular online newspaper websites: USAToday.com, NYTimes.com, DailyMail.co.uk, WashingtonPost.com, The-Guardian.com. A preliminary study of a subset of articles shows that articles regularly present a basic explanation of the science as well as some ethical concerns regarding (germ line) gene editing, including the potential benefits and harms. Studying media messages is important as they may influence public attitudes and debate and ultimately influence the acceptability (or lack thereof) for gene editing.

## C20.6

### Optimising CRISPR genome editing using machine learning

**R. Doyle, E1 2ST;**

Desktop Genetics, London, United Kingdom.

Decision rules are commonly used to select guide RNA sequences for a particular CRISPR genome editing application. Such rules use sequence-level and contextual features of the predicted cut site locations to predict the activity, specificity, and outcome of using CRISPR.

We designed a library of guides to test common rules for indel-mediated gene disruption. Our library maximized the orthogonality of the guide population, internally and with respect to previously published guides (rather than maximize guide performance), to create a better training data set for machine learning. We compared our machine-selected guides to a set of manually-selected guides, and tested different types of negative control guides. Our rules enriched for performant guides at a population level but could not select or eliminate individual guides with certainty. We find that combining decision rules has a cumulative effect; machine selection may be superior to manual; and using prior performance data and co-located genome features are critical.

We demonstrated significant sources of variance which impact guide activity and may confound statistical learning algorithms. We recommend: filtering guides using several rules, incorporation of genomic context, maximizing library orthogonality, inclusion of randomly chosen control guides, explicit incorporation of prior guide performance, and machine selection of guides.

## C21 Disorders with skin abnormalities

### C21.1

#### Antisense oligonucleotide-mediated exon skipping as a potential systemic treatment for recessive dystrophic epidermolysis bullosa

**J. Bremer<sup>1</sup>, O. Bornert<sup>2</sup>, A. Nyström<sup>2</sup>, A. H. Gostynski<sup>1</sup>, M. F. Jonkman<sup>1</sup>, A. Aartsma-Rus<sup>3</sup>, A. M. G. Pasmoij<sup>1</sup>, P. C. Van den Akker<sup>4,1</sup>;**

<sup>1</sup>Department of Dermatology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, <sup>2</sup>Department of Dermatology, Medical Center – University of Freiburg, Freiburg, Germany, <sup>3</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>4</sup>Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.



**Introduction:** Patients with the devastating skin disorder 'generalized severe' recessive dystrophic epidermolysis bullosa (RDEB) suffer lifelong blistering upon the slightest trauma and die of skin cancer before the age of 40. RDEB is caused by biallelic null-mutations in *COL7A1*, resulting in a complete absence of type VII collagen (ColVII). There is no cure for RDEB. Since 91% of *COL7A1* exons are in-frame and encode repetitive amino acid stretches, and low levels of ColVII already drastically improve the phenotype, *COL7A1* seems a perfect candidate for antisense oligonucleotide (AON)-mediated exon skipping therapy.

**Materials and Methods:** We examined the feasibility of AON-mediated exon skipping *in vitro* in primary cultured keratinocytes and fibroblasts, and *in vivo* in a human skin-graft mouse model after systemic delivery. The functionality of the resulting protein was assessed in diverse *in vitro* and *in vivo* functional assays.

**Results:** Treatment with AONs designed against a mutant exon 105 led to in-frame exon 105 skipping at the RNA level and restored ColVII expression *in vitro*. Moreover, systemic administration of these AONs to nude mice induced *in vivo* re-expression of ColVII in skin grafts, generated from mutant patient cells, engrafted on the mice's back. Additionally, the resulting ColVII proved functional *in vitro* in trypsin digestion, type IV collagen binding and fibroblast migration assays, and *in vivo* in a ColVII-deficient mouse model.

**Conclusions:** This study provides strong proof-of-concept for AON-mediated exon skipping as a systemic therapeutic strategy for RDEB.

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### C21.2

#### Osteoporosis in the segmental progeroid disorder gerodermia osteodysplastica is caused by defective decorin glycanation and TGF- $\beta$ induced oxidative stress.

**U. Kornak<sup>1</sup>, W. Chan<sup>1</sup>, M. Steiner<sup>1</sup>, J. Egerer<sup>1</sup>, A. C. Ma<sup>2</sup>, B. Busse<sup>3</sup>, S. Mizumoto<sup>4</sup>, J. Pestka<sup>3</sup>, H. Zhang<sup>1</sup>, I. Haussler<sup>5</sup>, K. Sugahara<sup>4</sup>, M. Amling<sup>2</sup>, D. Chan<sup>6</sup>, S. Mundlos<sup>1</sup>;**

<sup>1</sup>Charité-Universitätsmedizin Berlin, Berlin, Germany, <sup>2</sup>The University of Hong Kong, Hong Kong, China, <sup>3</sup>University Medical Center Hamburg-Eppendorf, Hamburg, Germany,

<sup>4</sup>Hokkaido University, Sapporo, Japan, <sup>5</sup>University Clinic Heidelberg, Heidelberg, Germany, <sup>6</sup>The University of Hong Kong, Hong Kong, China.

Age-related tissue deterioration is a multifactorial process that can be exemplarily studied in segmental progeroid disorders like gerodermia osteodysplastica (GO), a recessive condition characterized by skin laxity and early-onset osteoporosis. The GO disease gene GORAB, which encodes a small Golgi protein, was conditionally inactivated in mesenchymal progenitor cells of the limb skeleton. These GorabPrx1 mice showed hallmarks of age related bone changes including a disorganization of collagen fibrils and thinned, porous cortical bone culminating in spontaneous fractures of long bones. Reduced dermatan sulfate levels and impaired glycanation of decorin, indicating abnormal modification of extracellular matrix proteins, were associated with an increase of activated TGF- $\beta$  in tissues. Elevation of TGF- $\beta$  downstream signalling components included the reactive oxygen species (ROS) producing enzyme Nox4. The subsequent oxidative stress entailed an accumulation of DNA damage and cellular senescence in GorabPrx1 bone and in cultured human GO fibroblasts. Elevated ROS levels were also found after inactivation of gorab in a zebrafish model. Antioxidant treatment of GorabPrx1 mice ameliorated the osteoporosis phenotype underlining the importance of oxidative stress for the GO pathogenesis. Our data unravel a pathway involving Golgi function, matrix composition, TGF- $\beta$  signalling and oxidative stress in the pathogenesis of osteoporosis.

## C21.3

**Mutations in either TUBB or MAPRE2 cause circumferential skin creases Kunze type**

M. Isrie<sup>1</sup>, M. Breuss<sup>2</sup>, G. Tian<sup>3</sup>, A. H. Hansen<sup>2</sup>, F. Cristofoli<sup>1</sup>, J. Morandell<sup>2</sup>, E. Porta Dapena<sup>4</sup>, K. Leonard<sup>5</sup>, F. Tinsa<sup>6</sup>, S. Moortgat<sup>7</sup>, H. Ulucan<sup>8</sup>, E. Koparir<sup>9</sup>, E. Karaca<sup>9</sup>, N. Katsanis<sup>10</sup>, V. Marton<sup>11</sup>, J. R. Vermeesch<sup>1</sup>, E. Davis<sup>10</sup>, N. Cowan<sup>12</sup>, D. A. Keys<sup>2</sup>, H. Van Esch<sup>1</sup>:

<sup>1</sup>Center for Human Genetics, LEUVEN, Belgium, <sup>2</sup>Institute of Molecular Pathology, Vienna, Austria, <sup>3</sup>NYU Langone Medical Center, New York, NY, United States, <sup>4</sup>Ourense Hospital Complex, Ourense, Spain, <sup>5</sup>University of Alberta Stollery Children's Hospital, Edmonton, AB, Canada, <sup>6</sup>Children's Hospital of Tunis, Tunis, Tunisia, <sup>7</sup>Institut de Pathologie et de Génétique, Gosselies, Belgium, <sup>8</sup>Cerrahpasa Medical School of Istanbul University, Istanbul, Turkey, <sup>9</sup>Baylor College of Medicine, Houston, TX, United States, <sup>10</sup>Duke University, Durham, NC, United States, <sup>11</sup>The Arctic University of Norway, Tromso, Norway, <sup>12</sup>Center for Human Genetics, NYU Langone Medical Center, NY, United States.

Circumferential skin creases Kunze type (CSC-KT) is a specific congenital entity with an unknown genetic cause. The disease phenotype comprises characteristic circumferential skin creases accompanied by intellectual disability, cleft palate, a short stature and dysmorphic features. Here we report that mutations in either MAPRE2 or TUBB underlie the genetic origin of this syndrome. MAPRE2 encodes a member of the microtubule end-binding family of proteins that bind to the GTP cap at growing microtubule plus ends, while TUBB encodes a beta-tubulin isotype that is expressed abundantly in the developing brain. Functional analyses of the TUBB mutants show multiple defects in the chaperone-dependent tubulin heterodimer folding and assembly pathway that leads to a compromised yield of native heterodimers. The TUBB mutations also have an impact on microtubule dynamics in vivo. For MAPRE2, we show that the mutations result in enhanced MAPRE2 binding to microtubules, implying an increased dwell time at microtubule plus ends. Further, *in vivo* analysis of MAPRE2 mutations in a zebrafish model of craniofacial development show that the variants likely perturb the patterning of branchial arches, either through excessive activity (under a recessive paradigm) or through haploinsufficiency (dominant *de novo* paradigm). Taken together, our data add CSC-KT to the growing list of tubulinopathies, and highlight how multiple inheritance paradigms can affect dosage-sensitive biological systems so as to result in the same clinical defect.

## C21.4

**Two new genetic disorders presenting with lymphatic-related hydrops fetalis**

E. Fotiou<sup>1</sup>, S. Martin-Almedina<sup>1</sup>, I. Martinez-Corral<sup>2</sup>, R. Holdhus<sup>3</sup>, A. Vicente<sup>4</sup>, M. A. Simpson<sup>5</sup>, S. Lin<sup>6</sup>, K. Petersen<sup>3</sup>, A. Hoischen<sup>7</sup>, C. Gilissen<sup>7</sup>, H. Jeffery<sup>1</sup>, G. Atton<sup>1</sup>, J. W. Wiseman<sup>8</sup>, M. Wedin<sup>9</sup>, K. Gordon<sup>1</sup>, G. Brice<sup>1</sup>, I. Jeffery<sup>1</sup>, D. C. Rees<sup>9</sup>, C. Mignot<sup>10</sup>, J. Vogt<sup>11</sup>, T. Homfray<sup>1</sup>, M. P. Snyder<sup>6</sup>, S. G. Rockson<sup>6</sup>, S. Jeffery<sup>1</sup>, P. S. Mortimer<sup>1</sup>, S. Berland<sup>12</sup>, T. Makinen<sup>2</sup>, S. Mansour<sup>1</sup>, P. Ostergaard<sup>1</sup>:

<sup>1</sup>St George's University of London, London, United Kingdom, <sup>2</sup>Uppsala University, Uppsala, Sweden, <sup>3</sup>University of Bergen, Bergen, Norway, <sup>4</sup>University College London, London, United Kingdom, <sup>5</sup>King's College London School of Medicine, London, United Kingdom, <sup>6</sup>Stanford University, Stanford, CA, United States, <sup>7</sup>Radboud University Medical Center, Nijmegen, Netherlands, <sup>8</sup>AstraZeneca R&D, Molndal, Sweden, <sup>9</sup>King's College Hospital, London, United Kingdom, <sup>10</sup>St George's University of London Centre de Référence des Déficiences Intellectuelles de Causes Rares, Paris, France, <sup>11</sup>Birmingham Women's Hospital, Birmingham, United Kingdom, <sup>12</sup>Haukeland University Hospital, Bergen, Norway.

Hydrops fetalis describes fetal fluid accumulation in at least two of the following: the serous cavities or in body tissue. Previous studies have shown that non-immune hydrops fetalis is a heterogeneous condition and accounts for 85% of all cases of hydrops fetalis. A lymphatic abnormality is known to be the cause in approximately 15% of those, however, not much is known about the cause. The aim of this study was therefore to describe two inherited forms of lymphatic-related hydrops fetalis (LRHF); one autosomal dominant, the other autosomal recessive.

Exome sequencing on families, with a large number of *in utero* and neonatal deaths associated with non-immune hydrops fetalis, identified novel variants in the genes encoding Ephrin-receptor-B4 (EPHB4) (dominant form) and Piezo-type mechanosensitive ion channel component 1 (PIEZ01) (recessive form). Biochemical analysis of the mutant EPHB4 proteins demonstrate that they are devoid of tyrosine kinase activity. We further show that lymphatic endothelial specific inactivation of *Ephb4* in mouse embryos lead to subcutaneous edema due to defective lymphatic vessel formation. Mutations in PIEZ01 were also shown to affect expression of PIEZ01.

Together, these findings identify EPHB4 and PIEZ01 as critical regulators of early lymphatic vascular development and that mutations in these genes can cause two new forms of lymphatic-related non-immune hydrops fetalis with a high mortality. This also suggests not only is LRHF genetically heterogeneous, but it should also be considered in both dominant and recessive forms. This work was funded by British Heart Foundation (SP/13/5/30288) and Newlife Foundation for Disabled Children (12-13/01).

## C21.5

**Natural history of Ehlers-Danlos Syndrome (EDS) caused by CHST14/D4ST1 deficiency: from an international collaborative clinical study by the International Consortium for EDS**

T. Kosho<sup>1</sup>, D. Syk<sup>2</sup>, T. Van Damme<sup>2</sup>, H. Morisaki<sup>3</sup>, H. Kawame<sup>4</sup>, T. Sonoda<sup>5</sup>, Y. Hilhorst-Hofstee<sup>6</sup>, N. C. Voermans<sup>7</sup>, K. J. Wierenga<sup>8</sup>, R. Mendoza-Londono<sup>9</sup>, K. Ishikawa<sup>10</sup>, T. Kobayashi<sup>11</sup>, M. Kono<sup>11</sup>, K. Mochida<sup>12</sup>, K. Kikkawa<sup>13</sup>, C. Giunta<sup>14</sup>, A. R. Janecke<sup>15,16</sup>, N. Miyake<sup>17</sup>, F. Malfait<sup>18</sup>:

<sup>1</sup>Shinshu University School of Medicine, Matsumoto, Japan, <sup>2</sup>Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan,

<sup>4</sup>Division of Genomic Medicine Support and Genetic Counseling, Tohoku Medical Megabank Organization, Sendai, Miyagi, Japan, <sup>5</sup>Department of Pediatrics, University of Miyazaki, Miyazaki, Japan, <sup>6</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>7</sup>Department of Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, <sup>8</sup>Section of Genetics, Department of Pediatrics, the University of Oklahoma College of Medicine, Oklahoma, OK, United States, <sup>9</sup>Clinical and Metabolic Genetics, the Hospital for Sick Children, Toronto, ON, Canada, <sup>10</sup>Department of Pediatrics, Iwate medical University School of Medicine, Morioka, Japan, <sup>11</sup>Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan, <sup>12</sup>Department of Dermatology, University of Miyazaki, Miyazaki, Japan, <sup>13</sup>Department of Pediatrics, Kochi Health Science Center, Kochi, Japan, <sup>14</sup>Division of Metabolism, Connective Tissue Unit and Children's Research Center, University Children's Hospital, Zurich, Switzerland, <sup>15</sup>Department of Pediatrics I, Innsbruck Medical University, Innsbruck, Austria, <sup>16</sup>Division of Human Genetics, Innsbruck Medical University, Innsbruck, Austria, <sup>17</sup>Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Ehlers-Danlos syndrome (EDS) caused by CHST14/D4ST1 deficiency, also named as EDS musculocontractural type 1, is a recently-identified disorder with 38 published patients (25 families). We, on behalf of the International Consortium for EDS, have conducted an international collaborative study to establish the natural history. Comprehensive clinical information has been obtained from 20 published patients (17 families) and 15 additional patients (14 families). Patients had craniofacial characteristics in infancy (large fontanelle, hypertelorism, short/downslanting palpebral fissures, blue sclera, low-set and rotated ears, high arched palate, long philtrum, small mouth, micro-retrognathia) and from adolescence (slender, asymmetrical shape with protruding jaw), congenital multiple contractures (adducted thumbs, clubfoot), characteristic finger shapes (tapering, slender, cylindrical), progressive deformities of feet (pes planus with valgus) and spine (kypho/scoliosis), skin hyperextensibility, fragility, and bruising, wrinkling palmar creases, large subcutaneous hematomas (the most serious problem, occurring after minor traumas, spreading with severe pain, and sometimes accompanying hemorrhagic shock), ocular complications (retinal detachment), cryptorchidism in males, and other malformations (atrial septal defects). Two fatal events were described: a massive skin necrosis resulting from a large subcutaneous hematoma all around the leg; perforation of descending colon diverticula followed by skin rupture, with deterioration of general conditions to death. The disorder presents with an arthrogryposis-like appearance at birth and later fit the hallmark of EDS with a decrease of ADL/QOL and potential lethality due to progressive skeletal deformities, large subcutaneous hematomas, and visceral/ophthalmological complications. Grants: Practical Research Project for Rare/Intractable Diseases, Japan Agency for Medical Research and Development; Japan Foundation for Pediatric Research.

## C21.6

**Duplicated enhancer region upstream of the CTSB gene segregates with keratolytic winter erythema in South African and Norwegian families**

T. Ngcungcu<sup>1</sup>, M. Oti<sup>2</sup>, B. I. Haukanes<sup>3</sup>, J. C. Sitek<sup>4</sup>, F. Yang<sup>5</sup>, R. Brucolari<sup>6,5</sup>, T. Stokowy<sup>7</sup>, E. J. Oakley<sup>8</sup>, B. Linghu<sup>5</sup>, J. Zhu<sup>5</sup>, M. Sultan<sup>9</sup>, J. Schalkwijk<sup>9</sup>, C. von der Lippe<sup>4</sup>, H. G. Brunner<sup>9,10</sup>, S. Buechmann-Moller<sup>9</sup>, M. M. Thomas<sup>5</sup>, N. Nirmala<sup>5</sup>, H. van Bokhoven<sup>9</sup>, V. M. Steen<sup>7</sup>, P. R. Hull<sup>11</sup>, J. Szustakowski<sup>12</sup>, F. Staedtler<sup>9</sup>, H. Zhou<sup>2</sup>, T. Fiskerstrand<sup>1</sup>, M. Ramsay<sup>1</sup>:

<sup>1</sup>University of the Witwatersrand, Johannesburg, South Africa, <sup>2</sup>Radboud University, Nijmegen, Netherlands, <sup>3</sup>Haukeland University Hospital, Bergen, Norway, <sup>4</sup>Oslo University Hospital, Oslo, Norway, <sup>5</sup>Novartis Institutes for BioMedical Research, Cambridge, MA, United States, <sup>6</sup>Congenomics, Glastonbury, CT, United States, <sup>7</sup>University of Bergen, Bergen, Norway, <sup>8</sup>Novartis Institutes for BioMedical Research, Basel, Switzerland, <sup>9</sup>Radboud University Medical Center, Nijmegen, Netherlands, <sup>10</sup>Maastricht University Medical Center+, Maastricht, Netherlands, <sup>11</sup>Dalhousie University, Halifax, NS, Canada, <sup>12</sup>Bristol-Myers Squibb, Princeton, NJ, United States.

Keratolytic winter erythema (KWE) is a rare autosomal dominant skin disorder characterized by recurrent episodes of palmo-plantar centrifugal erythema and epidermal peeling. KWE was previously mapped to 8p23-p22 (KWE critical region) in South African families, who are expected to share the same mutation due to a founder effect. Using targeted resequencing of the KWE critical region in three South African families and SNP array/whole genome sequencing in two Norwegian families, we identified two overlap-

ping tandem duplications of 7.67 kb and a 15.93 kb respectively. The duplications are located upstream to the *CTSB* gene, and the 2.63 kb overlapping region includes an enhancer element that is active in keratinocytes. Based on NHEK cell line Hi-C data and MCF-7 cell line CTCF ChIA-PET data, three potential topological domains were revealed, all containing the enhancer and *CTSB*, plus either *FDFT1* and even *NEIL2*. However, the activity of the enhancer correlated with *CTSB* but not with *FDFT1* and *NEIL2* gene expression in differentiating keratinocytes and other cell lines, and MCF-7 RNAPII ChIA-PET data showed that the enhancer interacts with the *CTSB* promoter but not with the *FDFT1* or *NEIL2* promoters, suggesting that the enhancer normally regulates *CTSB* expression. In conclusion, we show that KWE in South African and Norwegian families is caused by tandem duplications in non-coding DNA containing an active enhancer element for *CTSB*. Duplication of this region may cause aberrant expression of nearby genes due to changes in the topological domain structure, in response to environmental triggers.

## C22 Cardiovascular disorders

### C22.1

#### FOXF2, a novel risk locus for stroke and small artery disease: a genome-wide association study

**G. Chauhan**<sup>1</sup>, C. R. Arnold<sup>2</sup>, A. Y. Chu<sup>3</sup>, M. Fornage<sup>4</sup>, A. Reyahi<sup>5</sup>, J. C. Bis<sup>6</sup>, A. S. Havulinna<sup>7</sup>, the Cervical Artery Dissection and Ischemic Stroke Patients (CADISP) study, the METASTROKE consortium, the Stroke Genetics Network (SiGN), the International Stroke Genetics Consortium (ISGC), O. J. Lehmann<sup>8</sup>, L. J. Launer<sup>9</sup>, M. Ikram<sup>10</sup>, P. Carlsson<sup>3</sup>, D. I. Chasman<sup>3</sup>, S. J. Childs<sup>2</sup>, W. T. Longstreth, Jr<sup>11</sup>, S. Seshadri<sup>12</sup>, S. Debette<sup>1</sup>, the Neurology Working Group of the CHARGE consortium;

<sup>1</sup>Université de Bordeaux, Centre Inserm U 897, Bordeaux, France, <sup>2</sup>Alberta Children's Hospital Research Institute and Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada, <sup>3</sup>Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA, United States, <sup>4</sup>Institute of Molecular Medicine and Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, United States, <sup>5</sup>Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden, <sup>6</sup>Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, United States, <sup>7</sup>National Institute for Health and Welfare, Helsinki, Finland, <sup>8</sup>Department of Ophthalmology, University of Alberta, Edmonton, AB, Canada, <sup>9</sup>IRP/NIA/NIH, MD, USA, Bethesda, MD, United States, <sup>10</sup>Department of Epidemiology, Erasmus MC, Rotterdam, Netherlands, <sup>11</sup>Department of Epidemiology, University of Washington, Seattle, WA, United States, <sup>12</sup>Department of Neurology, Boston University School of Medicine, Boston, MA, United States.

**Background:** Genetic determinants of stroke, the leading neurological cause of death and disability, are poorly understood and have seldom been explored in the general population.

**Methods:** We performed a genome-wide screen for common genetic variants associated with incident stroke risk in 18 prospective population-based cohorts comprising 84,961 participants, of whom 4,348 experienced stroke. We followed-up variants with  $p < 5 \times 10^{-6}$  in the largest available cross-sectional studies (70,804 participants of whom 19,816 experienced stroke). For genome-wide significant findings ( $p < 5 \times 10^{-8}$ ), we explored associations with additional cerebrovascular phenotypes and undertook functional analyses in mouse and zebrafish mutants.

**Results:** We replicated seven of eight known loci for ischemic stroke and identified a novel locus at chr6p25 (rs12204590, near *FOXF2*) associated with risk of all stroke: odds ratio (OR) = 1.08 (95%CI:1.05-1.12),  $p = 1.48 \times 10^{-8}$  (minor allele frequency 21%). The rs12204590 stroke risk allele also increased MRI-defined white matter hyperintensity (WMH) burden, a marker of cerebral small artery disease, in stroke-free adults ( $N = 21,079$ ;  $p = 0.0025$ ). Consistently, young patients with segmental deletions of *FOXF2* exhibited extensive WMH burden. Conditional (inducible) deletion of *Foxf2* in adult mice resulted in cerebral infarction, reactive gliosis, and microhemorrhage. The zebrafish equivalents of *FOXF2* (orthologs) *foxf2b/foxf2a* were expressed in brain pericytes and mutant *foxf2b/-* cerebral vessels showed decreased smooth muscle cell and pericyte coverage.

**Conclusion:** In our study of 155,765 persons in total (24,164 with stroke), we identified common variants near *FOXF2* associated with increased stroke susceptibility. Extensive epidemiological and experimental data suggest that *FOXF2* mediates this association, potentially via differentiation defects of cerebral vascular mural cells.

### C22.2

#### Whole exome sequencing identifies ALPK3 as a new disease gene causing both severe paediatric and 'milder' adult-onset cardiomyopathies.

**J. D. H. Jongbloed**<sup>1</sup>, J. M. A. Verhagen<sup>2</sup>, R. Almomani<sup>3</sup>, E. Brosens<sup>2</sup>, K. Y. van Spaendonck-Zwarts<sup>1,3</sup>, A. Asimaki<sup>4</sup>, P. A. van der Zwaag<sup>1</sup>, I. M. E. Frohn-Mulder<sup>5</sup>, A. M. Bertoli-Avella<sup>2,6</sup>, L. G. Boven<sup>1</sup>, M. A. van Slegtenhorst<sup>2</sup>, J. J. van der Smagt<sup>2</sup>, W. F. J. van IJcken<sup>6</sup>, B. Timmer<sup>9</sup>, M. van Stuijvenberg<sup>10</sup>, R. M. Verdijk<sup>11</sup>, J. E. Saffitz<sup>12</sup>, F. A. du Plessis<sup>5</sup>, M. Michels<sup>12</sup>, M. P. van den Berg<sup>13</sup>, R. M. W. Hofstra<sup>2</sup>, P. van der Harst<sup>13</sup>, R. J. Sinke<sup>1</sup>, P. van Tintelen<sup>1,3</sup>, M. W. Wessels<sup>2</sup>, **J. C. Herkert**<sup>1</sup>, I. M. B. H. van de Laar<sup>2</sup>,

<sup>1</sup>Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, <sup>2</sup>Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands, <sup>3</sup>Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands, <sup>4</sup>Department of Pathology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, United States, <sup>5</sup>Department of Paediatric Cardiology, Erasmus University Medical Center, Rotterdam, Netherlands,

<sup>6</sup>Centogene AG, Rostock, Germany, <sup>7</sup>Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands, <sup>8</sup>Center for Biomics, Erasmus University Medical Center, Rotterdam, Netherlands, <sup>9</sup>Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands,

<sup>10</sup>Division of Neonatology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, <sup>11</sup>Department of Pathology, Erasmus University Medical Center, Rotterdam, Netherlands, <sup>12</sup>Department of Cardiology, Erasmus University Medical Center, Rotterdam, Netherlands, <sup>13</sup>Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Paediatric cardiomyopathies are a heterogeneous group of disorders characterized by structural and functional cardiac abnormalities. Up to 40% of affected children die or undergo cardiac transplantation within five years of diagnosis. While understanding the molecular basis of paediatric cardiomyopathy has greatly improved over the last decades, in many cases the underlying cause remains unknown. Currently available (targeted/custom based) diagnostic tests do not represent the whole spectrum of genetic etiologies and lead to a genetic diagnosis in a subset of paediatric patients only. Using homozygosity mapping and exome sequencing in two consanguineous families with idiopathic paediatric cardiomyopathy, we identified homozygous truncating mutations in a new disease gene: alpha-kinase 3 (ALPK3). This gene encodes a nuclear kinase essential for early differentiation of cardiomyocytes, being involved in important transcription factor pathways. A third family carrying mutated ALPK3 was identified upon cohort screening. Patients with biallelic mutations presented with severe cardiomyopathy leading to early lethality or biventricular dysfunction in childhood. Some heterozygous family members showed adult-onset cardiomyopathy with atypically distributed hypertrophy, indicating that this gene may also play a role in dominantly inherited cardiomyopathies. We provide microscopic evidence of intercalated disc remodelling, as previously observed in Alpk3 knockout mice. Further functional experiments and studies on the role of the gene in adult-onset cardiomyopathies and cardiac hypertrophy are ongoing.

In conclusion, biallelic truncating mutations in ALPK3 cause severe paediatric cardiomyopathy. Our findings highlight the importance of transcription factor pathways in the molecular mechanisms underlying human cardiomyopathies and underscore the high genetic heterogeneity of paediatric cardiomyopathies.

### C22.3

#### Exome sequencing reveals distinct genetic architectures for syndromic and nonsyndromic congenital heart defects

**A. Sifrim**<sup>1</sup>, M. Hitz<sup>1,2</sup>, A. Wilsdon<sup>3</sup>, J. Breckpot<sup>4</sup>, S. H. Al Turk<sup>5</sup>, B. Thienpont<sup>6,7</sup>, J. McRae<sup>1</sup>, T. W. Fitzgerald<sup>1</sup>, T. Singh<sup>1</sup>, G. J. Swaminathan<sup>1</sup>, D. Rajan<sup>1</sup>, E. Prigmore<sup>1</sup>, The INTERVAL study, The UK10K Consortium, Competence Network for Congenital Heart Defects, German Register for Congenital Heart Defects, S. Mital<sup>8</sup>, P. Daubene<sup>9</sup>, B. Keavney<sup>10</sup>, J. Goodship<sup>11</sup>, R. M. Abu-Sulaiman<sup>12,13</sup>, S. Klaassen<sup>14,15</sup>, C. F. Wright<sup>1</sup>, H. V. Firth<sup>16</sup>, J. C. Barrett<sup>1</sup>, K. Devriendt<sup>1</sup>, D. R. Fitzpatrick<sup>17</sup>, J. D. Brook<sup>3</sup>, The Deciphering Developmental Disorders Study, M. E. Hurles<sup>1</sup>,

<sup>1</sup>Wellcome Trust Sanger Institute, Hinxton, United Kingdom, <sup>2</sup>UKSH Kiel, Kiel, Germany,

<sup>3</sup>University of Nottingham, Nottingham, United Kingdom, <sup>4</sup>University Hospitals Leuven, Leuven, Belgium, <sup>5</sup>Harvard Medical School Genetics Training Program, Boston, MA, United States, <sup>6</sup>Vesalius Research Center, Flemish Institute for Biotechnology, Leuven, Belgium, <sup>7</sup>Katholieke Universiteit Leuven, Leuven, Belgium, <sup>8</sup>Hospital for Sick Children, Toronto, ON, Canada, <sup>9</sup>Royal Brompton Hospital, London, United Kingdom, <sup>10</sup>University of Manchester, Manchester, United Kingdom, <sup>11</sup>Newcastle University, Newcastle, United Kingdom, <sup>12</sup>Ministry of National Guard-Health Affairs, Riyadh, Saudi Arabia, <sup>13</sup>King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia, <sup>14</sup>Charite Medical Faculty and Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, <sup>15</sup>German Heart Institute Berlin, Berlin, Germany, <sup>16</sup>East Anglian Medical Genetics, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, <sup>17</sup>MRC Institute of Genetics and Molecular Medicine (IGMM), University of Edinburgh, Edinburgh, United Kingdom.

Congenital Heart Defects (CHD) have a neonatal incidence of 0.8-1%. Despite abundant examples of monogenic CHD in humans and mice, CHD has

a low absolute sibling recurrence risk (~2.7%), suggesting a considerable role for de novo mutations, and/or incomplete penetrance. De novo protein-truncating variants have been shown to be enriched among the 10% of 'syndromic' patients with extra-cardiac manifestations (Homsy et al., 2015). However, these findings do not explain the recurrence risk in the majority (~90%) of CHD cases which present themselves clinically as isolated cardiac defects. We exome sequenced 4,593 individuals from 1,823 CHD families (1,365 trios, 32 multi-sibling families and 458 singletons), including both syndromic (n=610) and non-syndromic cases (n=1,281). In the syndromic CHD cohort, we confirmed a significant enrichment of de novo, but not inherited, protein-truncating variants, in known CHD-associated genes, consistent with recent findings. Conversely, in non-syndromic CHD we observed significant enrichment of protein-truncating variants inherited from unaffected parents in known CHD genes. These findings point towards a role for variants with reduced penetrance in these non-syndromic cases. Additionally, we identified three novel genome-wide significant syndromic CHD disorders caused by de novo mutations in CHD4, CDK13 and PRKD1. To summarise, our study reveals distinct genetic architectures underlying the low sibling recurrence risk in syndromic and non-syndromic CHD.

#### C22.4

#### Defective connective tissue remodeling in Smad3 mice leads to accelerated aneurysmal growth through disturbed downstream TGF-β signaling

I. van der Pluijm, N. van Vliet, J. von der Thussen, J. Robertus, Y. Ridwan, P. van Heijningen, B. van Thiel, M. Vermeij, R. Buijs-Offerman, R. Kanaar, A. Bertoli-Avella, J. Eisers; Erasmus Medical Center, Rotterdam, Netherlands.

Aneurysm-osteoarthritis syndrome (AOS, or Loeys-Dietz syndrome type3, LDS3) is characterized by aortic aneurysms and early-onset osteoarthritis, caused by SMAD3 mutations. Smad3 is part of the Smad2/3/4 transcription factor, essential for TGF-β-activated transcription. Although mutations in TGF-β-related genes result in aneurysm formation, the underlying mechanism is unknown. Here, we examined aneurysm formation and progression in Smad3<sup>-/-</sup> animals.

Smad3<sup>-/-</sup> animals developed aortic aneurysms rapidly, resulting in premature death, earlier in males than females. Aortic wall immunohistochemistry showed no increase in extracellular matrix and collagen, nor loss of vascular smooth muscle cells (VSMCs) but instead revealed medial elastin disruption and adventitial inflammation. Remarkably, matrix metalloproteases (MMPs) were not activated in VSMCs, but were specifically activated in inflammatory areas. Although Smad3<sup>-/-</sup> aortas showed increased nuclear pSmad2 and pErk, indicative of TGF-β receptor activation, downstream TGF-β-activated genes were not upregulated. Increased pSmad2 and pErk staining in pre-aneurysmal Smad3<sup>-/-</sup> aortas without immune infiltrates implied that the aortic damage and subsequent TGF-β receptor-activated signaling precede aortic inflammation. Finally, increased pErk together with impaired downstream TGF-β-activated transcription resulted in increased Smad3<sup>-/-</sup> VSMC proliferation.

Smad3<sup>-/-</sup> animals recapitulate the aortic phenotype observed in patients with SMAD3 mutations. Smad3 deficiency leads to imbalanced activation of downstream genes and no activation of MMPs in VSMCs, causing an immune response resulting in rapid dilatation and rupture of the aortic wall. Our findings uncover new possibilities for treatment of patients carrying SMAD3 mutations; instead of treating with drugs targeting the TGF-β pathway, they may benefit more from immune suppression.

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#### C22.5

#### Loss-of-function mutations in the X-linked gene BGN cause a severe syndromic form of thoracic aortic aneurysms and dissections

J. A. N. Meester<sup>1</sup>, G. Vandeweyer<sup>1</sup>, I. Pintelon<sup>2</sup>, K. Waitzman<sup>3</sup>, L. Young<sup>3</sup>, L. W. Markham<sup>4</sup>, J. Vogt<sup>5</sup>, J. Richer<sup>6</sup>, L. M. Beauchesne<sup>7</sup>, S. Unger<sup>8</sup>, A. Superti-Furga<sup>9</sup>, E. Reyniers<sup>1</sup>, A. Verstraeten<sup>1</sup>, L. Van Laer<sup>1</sup>, B. L. Loey<sup>1</sup>;

<sup>1</sup>Center of Medical Genetics, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium, <sup>2</sup>Department of Cell Biology and Histology, University of Antwerp, Antwerp, Belgium, <sup>3</sup>Department of Pediatric Cardiology, Lurie Children's Hospital of Chicago, Chicago, IL, United States, <sup>4</sup>Divisions of Pediatric and Adult Cardiology, Vanderbilt University, Nashville, TN, United States, <sup>5</sup>Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom, <sup>6</sup>Department of Medical Genetics, University of Ottawa Heart Institute, Ottawa, ON, United States, <sup>7</sup>Division of Cardiology, University of Ottawa Heart Institute, Ottawa, ON, United States, <sup>8</sup>Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, <sup>9</sup>Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Thoracic aortic aneurysm and dissection (TAAD) is typically inherited in an

autosomal dominant manner but rare X-linked families have been described. So far, FLNA is the only X-linked gene associated with a syndromic form of TAAD, namely the periventricular nodular heterotopia type of Ehlers-Danlos syndrome. However, FLNA only explains a small number of the X-linked TAAD families.

We performed targeted resequencing of nearly 500 extracellular matrix and TGFβ related genes in a cohort of 11 Marfan-like probands without known causal mutation. We identified two patients with loss-of-function mutations in BGN, encoding the extracellular matrix small leucine-rich proteoglycan biglycan. Subsequent Sanger sequencing of BGN in 400 male and 200 female TAAD-patients, negative for known TAAD genes, identified a splice site mutation in a male proband and suggested a deletion in two other male probands. The latter were confirmed by micro-array analysis. The clinical phenotype is characterized by early onset aortic aneurysm (age 1 year) and dissection (age 15 years). Other recurrent findings include hypertelorism, pectus deformity, joint hypermobility, contractures and mild skeletal dysplasia. Histological stainings of the patients' aortic wall revealed a substantial reduction in collagen content, while elastin fibers appeared normal. Biglycan deficiency in male BALB/cA mice leads to sudden death from aortic rupture, indicating that biglycan is both structurally and functionally essential for the integrity of the aortic wall.

These results confirm that BGN gene defects in human cause an X-linked syndromic form of severe TAAD.

## C23 Functional Genomics

#### C23.1

#### Mosaic loss of chromosome Y (LOY) in peripheral blood is associated with age, smoking, shorter survival and increased risk of cancer and Alzheimer's disease (AD) in men

J. Dumanski<sup>1,2</sup>, J. Lambert<sup>3</sup>, C. Rasi<sup>1,2</sup>, V. Giedraitis<sup>4</sup>, H. Davies<sup>1,2</sup>, B. Grenier-Boley<sup>3</sup>, C. M. Lindgren<sup>5,6</sup>, D. Campion<sup>7</sup>, C. Dufouil<sup>8</sup>, C. Bellenguez<sup>9</sup>, V. Chouraki<sup>9</sup>, A. Brice<sup>10</sup>, J. Epelbaum<sup>11</sup>, C. Berr<sup>12</sup>, J. Dartigues<sup>13</sup>, D. Hannequin<sup>7</sup>, A. Boland<sup>13</sup>, M. Lathrop<sup>14</sup>, D. Zelenika<sup>13</sup>, F. Pasquier<sup>9,15</sup>, P. Amouyel<sup>9,15</sup>, L. Lannfelt<sup>4</sup>, M. Ingelsson<sup>4</sup>, L. Kilander<sup>4</sup>, L. Lind<sup>16</sup>, L. A. Forssberg<sup>12</sup>;

<sup>1</sup>Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, <sup>2</sup>Science for Life Laboratory, Uppsala, Sweden, <sup>3</sup>Institut Pasteur de Lille, Université de Lille, Lille, France, <sup>4</sup>Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden, <sup>5</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, <sup>6</sup>Broad Institute of MIT and Harvard University, Cambridge, MA, United States, <sup>7</sup>Rouen University Hospital, Rouen, France, <sup>8</sup>Victor Segalen University, Bordeaux, France, <sup>9</sup>Université de Lille, Lille, France,

<sup>10</sup>Université Pierre et Marie Curie, Paris, France, <sup>11</sup>Université Paris Descartes, Paris, France, <sup>12</sup>Hôpital La Colombière, Montpellier, France, <sup>13</sup>Institut de Génomique, Evry, France, <sup>14</sup>Université McGill, Montréal, QC, Canada, <sup>15</sup>Centre Hospitalier Régional Universitaire de Lille, Lille, France, <sup>16</sup>Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

**Introduction:** Life expectancy of men is about 5 years shorter compared with women but the underlying factor(s) have been elusive. Our previous results show that mosaic loss of chromosome Y in blood cells (LOY) is associated with age, smoking, all-cause mortality and non-hematological tumors (Nat. Genet. 2014 PMID:24777449, Science 2015 PMID:25477213). The aim of the present study was to test if men with LOY are more susceptible to Alzheimer's disease (AD).

**Materials and Methods:** LOY in blood cells was estimated using SNP-array data from >3200 men from one AD case-control study and two prospective cohorts. Whole genome sequencing was used for validations. A set of statistical techniques were used to evaluate association between LOY in blood and AD diagnosis.

**Results:** LOY was detected in ~17% of participants (median age=73, range=37-96). We found that men with AD diagnosis had a higher degree of LOY mosaicism in the case-control study (adjusted odds ratio=2.80, AD events=606, p=0.0184). Furthermore, analysis of the two prospective cohorts showed that men with LOY in blood at sampling had an increased risk for incident AD during follow-up time (HR=6.80, 95% CI=2.16-21.43, AD events=140, p=0.0011).

**Conclusions:** Our results suggest that LOY in blood cells is associated with increased risk for both AD and cancer, suggesting a role of LOY in blood cells on disease processes in other tissues, possibly via defective immunosurveillance functions of immune cells without the Y chromosome. Hence, as a male-specific genetic risk factor, LOY might help explain why males on average live shorter than females.


**C23.2**

### Single cell allele specific expression (ASE) in T21 and common trisomies: Novel approach to understand gene dosage effects in Down syndrome and common aneuploidies

**G. Stamoulis<sup>1</sup>, F. Santoni<sup>2</sup>, P. Makrythanasis<sup>2</sup>, A. Letourneau<sup>1</sup>, M. Guipponi<sup>2</sup>, M. Garieri<sup>1</sup>, N. Panousis<sup>1</sup>, E. Falconnet<sup>1</sup>, P. Ribaux<sup>1</sup>, C. Borel<sup>1</sup>, S. E. Antonarakis<sup>1,2,3</sup>;**

<sup>1</sup>Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland, <sup>2</sup>Geneva University Hospitals-HUG, Service of Genetic Medicine, 1211 Geneva 4, Switzerland, Geneva, Switzerland, <sup>3</sup>IGE3 Institute of Genetics and Genomics of Geneva, University of Geneva, 1211, Geneva, Switzerland.

Trisomy 21(T21), is a model disorder of altered gene expression. We have previously used a pair of monozygotic-twins discordant for T21 to study the global dysregulation of gene expression, eliminating genetic variation among individuals (Nature:508;345-350;2014). Majority of previous studies on aneuploidies were conducted on cell-populations or tissues. Studies on gene and allelic-expression of single-cells(SC) may reveal important biological insights regarding the cellular impact of aneuploidy elucidating the fundamental mechanisms of gene dosage. We estimated the allele-specific-expression(ASE) from RNAseq of ~1000 single-cells in different aneuploidies:352 SC fibroblasts (172 Normal-179 T21 cells) from the pair of monozygotic-twins discordant for T21, 166 from a mosaic-T21, 176 mosaic-T18, 151 mosaic-T8, and 146 SC-fibroblasts from mosaic-T13. In the monozygotic-twins, considerable number of heterozygous sites genome-wide were expressed monoallelically (Normal:73.5%-564,668 observations, and T21:78.7%-549,799 observations). There was considerable monoallelic-expression for chr21 sites in Normal and, surprisingly, in T21 cells as well (Normal:63.3%-5,009 observations, T21:72.8%-6,456 observations). We classified chr21 genes in three classes according to the level of the aggregate monoallelic-expression of their corresponding sites (9-monoallelic, 29-intermediate, 2-biallelic). We hypothesize that each class of genes contributes in a specific way to the phenotypic variability of Down-Syndrome(DS). Similar results, (i.e. extensive monoallelic-expression of genes on the supernumerary chromosomes, were also observed in the other aneuploidies. Our analysis demonstrated that, for genes with monoallelic-expression, the altered gene dosage induced by the aneuploid chromosome is due to the number of cells expressing the gene on the supernumerary chromosome. This difference in the fraction of expressing cells could contribute to the development and variability of phenotypes in aneuploidies. This study provides a new fundamental understanding of gene dosage effects in aneuploidies.


**C23.3**

### The landscape of polymorphic inversions and their functional impact in the human genome

**J. Lerga-Jaso<sup>1</sup>, M. Oliva<sup>1</sup>, S. Villatoro<sup>1</sup>, D. Izquierdo<sup>1</sup>, L. Pantano<sup>1</sup>, S. Casillas<sup>1,2</sup>, M. Cáceres<sup>1,3</sup>;**

<sup>1</sup>Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, <sup>2</sup>Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, <sup>3</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Recent advances in genomic techniques have generated an increasing interest in structural variants (SVs). However, there is still limited data on their functional impact. This is particularly true for inversions, in which their balanced nature and the complex repetitive regions where they appear make their study especially challenging. In this regard, we have designed a null model to elucidate the functional characteristics of these variants, taking as reference the most reliable set of human polymorphic inversions so far from the InvFEST database. Human inversions show the signature of natural selection, avoiding more often than expected not only genes, but also other functional elements such as enhancers, chromatin domain boundaries and highly conserved regions. Moreover, we measured the effect of 45 experimentally-genotyped polymorphic inversions on gene expression based on the transcriptome data from lymphoblastoid cell lines of 175 individuals from the Geuvadis project. Using a combination of well-established tools and further filtering criteria to minimize false positives, we found that although the majority of inversions do not appear to have any significant regulatory effect in this cell type, around half a dozen of them have a clear influence on specific genes, both in cis and in trans. Finally, we intersected SNPs reported as GWAS hits of different human phenotypes with the inversions in our dataset. All together, these results illustrate the potential impact of inversions on the human genome, and contribute to shed light on the molecular mechanisms responsible for phenotypic variability.

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**C23.4**

### Assessment of the GENCODE annotation through experimental validation

**A. Ferreira<sup>1,2</sup>, B. Uszczynska<sup>3</sup>, J. Lagarde<sup>3</sup>, J. Chrast<sup>1</sup>, A. Frankish<sup>4</sup>, J. Harrow<sup>4</sup>, R. Guigó<sup>3</sup>, A. Reymond<sup>1</sup>;**

<sup>1</sup>Center for Integrative Genomics, Lausanne, Switzerland, <sup>2</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland, <sup>3</sup>Centre for Genomic Regulation, Barcelona, Spain, <sup>4</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Within the ENCODE consortium, GENCODE aims to accurately annotate all protein-coding genes, pseudogenes and non-coding transcribed loci in the human genome through manual curation and computational analysis. Lower confidence transcribed loci, i.e. not supported by ENCODE or GTEx RNA-seq data, were systematically experimentally evaluated in 8 tissues by RT-PCR amplification followed by highly multiplexed sequencing readout, a method we coined RT-PCR-seq. 78% of all assessed junctions are confirmed by this evaluation procedure demonstrating the high quality of the annotation reached by the GENCODE gene set. In a second phase unvalidated junctions were reevaluated by RT-PCR-seq in a second set of 8 tissues and with a novel pair of primers to account for tissue specificity and bad design of the oligos, respectively.

To assess the completion of the GENCODE annotation we then tried to amplify new exons and/or new splice junctions of 541 deeply annotated protein-coding genes that belong to the UK Genetic Testing Network list through individual 5' and 3' nested-RACEs in 8 different tissues (brain, testis, heart, kidney, liver, lung, spleen and skeletal muscle) paired with long-range Pacific Biosciences sequencing. Whereas these experiments failed to reveal a large number of new exons and splice sites, they further contributed to the improvement of the human genome annotation through identification of new splice junctions between already annotated exons, de facto new transcripts. While the results demonstrate that the human transcriptome annotation is far from complete, we are moving to accurately identifying all protein-coding loci of major interest to the clinical community.


**C23.5**

### Single-cell RNA-seq analysis of human pancreatic islets

**M. Garieri<sup>1,2</sup>, C. Borel<sup>1</sup>, T. Stoeger<sup>3</sup>, F. Santoni<sup>1</sup>, C. Howald<sup>1,2</sup>, P. Ribaux<sup>1</sup>, L. Pelkmans<sup>3</sup>, P. Halban<sup>1</sup>, S. Antonarakis<sup>4</sup>, E. Dermitzakis<sup>1,2</sup>;**

<sup>1</sup>Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland, <sup>2</sup>Swiss Institute of Bioinformatics (SIB), Geneva, Switzerland, <sup>3</sup>Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

Pancreatic islets consist of several endocrine cell types. We investigate the heterogeneity of this tissue using single-cell (SC) RNA-seq. Dissociated islet cells of 3 donors (n=241, 183 after quality control) were analyzed. We also sequenced sorted-beta SCs (n=60, 44 after quality control) from the same individuals.

Clustering analysis on islet SCs identified the follow populations of cells: beta SCs (mean INS expression 40777 RPKM, representing the 75% of all SCs), alpha (mean GCG expression 86443 RPKM, 12%), gamma (mean PPY expression 49928 RPKM, 3%), delta (mean SST expression 76061 RPKM, 2%) and a small population of exocrine SCs. In the sorted beta SCs we observed beta SCs (INS 32708 RPKM, 93%) and delta SCs (SST 135327 RPKM, 7%).

Interestingly we observed that insulin (INS) is expressed in all the islets SCs and not just in the subpopulation of beta SCs, in addition GCG has expression in 93% of all islets SCs. We controlled for the absence of transcript contamination of INS and GCG by sequencing 11 empty chambers. We are verifying the cellularity of the cell types and the presence of INS and GCG transcript in most of the SCs using another technology (branched-DNA single-molecule FISH) allowing for quantification and cellular localization of transcripts.

Differential expression and differential splicing analysis has been performed among all different cell types.

SC transcriptome analyses provide an unprecedented opportunity to discover the heterogeneity of human pancreatic islets of Langerhans.

M.G and C.B. and the labs of SEA and ETD contributed equally to the work.


**C23.6**

### Identifying novel long non-coding RNAs in the Human genome

**M. P. Hardy<sup>1</sup>, J. Harrow<sup>1</sup>, J. Loveland<sup>1</sup>, A. Frankish<sup>1</sup>, HAVANA Group; Wellcome Trust Sanger Institute, Cambridge, United Kingdom.**

Multiple lines of evidence are emerging that long non-coding RNAs (lncRNAs) can be crucial regulators and markers of disease. For example, the long intergenic RNA (lincRNA) MALAT1 has been shown to regulate cancer tumor growth in bone and has been identified as a potential therapeutic target in osteosarcoma\*. As part of the GENCODE project, the HAVANA team at the Wellcome Trust Sanger Institute aims to identify and manually annotate all lncRNAs in the human and mouse genomes. Our most recent release for

human (GENCODE 24) identifies 15,941 lncRNA loci containing 28,031 transcripts. Historically, lncRNA annotation has been supported by ESTs and cDNAs submitted to INSDC databases. Recent developments in next generation sequencing technology provide a huge amount of transcriptome data, however, this data needs to be handled carefully if it is to be used for building high quality gene annotation\*\*. We will discuss the incorporation of public RNAseq and SLRseq datasets into our annotation pipeline to identify novel loci and describe our PacBio Capture-seq pipeline to extend loci to their full length and identify alternative splicing (AS). Additionally, we will show how we use PhyloCSF and Mass Spectrometry data to check coding potential in lncRNA loci and confirm their non-coding status, or update their annotation. Combining multiple public and bespoke next-generation data sets allows us to identify novel lncRNA loci, extend and add AS transcripts to existing loci and validate their non-coding annotation in an effort to provide a more detailed picture of the human non-coding transcriptome.

\*PubMedID 25504755

\*\*<http://www.gencodegenes.org/rgasp/>

## C24 Rare Disease Gene Discoveries

### C24.1

#### Studying the genetic basis of idiopathic short stature using whole exome sequencing

C. Thiel<sup>1</sup>, N. N. Hauer<sup>1</sup>, E. Schöller<sup>1</sup>, S. Schuhman<sup>1</sup>, M. T. Wittmann<sup>1</sup>, B. Popp<sup>1</sup>, S. Uebe<sup>1</sup>, C. Büttner<sup>1</sup>, A. B. Ekici<sup>1</sup>, K. Kutsche<sup>2</sup>, D. Wieczorek<sup>3</sup>, H. Sticht<sup>4</sup>, H. Dörr<sup>5</sup>, A. Reis<sup>1</sup>, <sup>1</sup>Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany, <sup>2</sup>Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, <sup>3</sup>Institute of Human-Genetics, University of Düsseldorf, Düsseldorf, Germany, <sup>4</sup>Institute of Biochemistry, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany, <sup>5</sup>Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.

Shortness of stature is a common medical concern in childhood with an incidence of 3%. After excluding common defects the underlying cause remains unknown in approximately 70-80%.

We now recruited and clinically characterized a group of >500 families with idiopathic short stature (ISS) and selected 200 individuals where common genetic causes were excluded for whole exome sequencing (100 Trios, 100 affected only).

Surprisingly, in 21 Trios we found mutations in known short stature genes where characteristic clinical features of the syndromes were missing. In 62 of the remaining 89 trios we found potential protein affecting variants in 126 novel candidate genes. A second variant was found for 4 of these 126 candidate genes in the affected only analysis. 2 further genes were recently reported for ISS in agreement with a dominant de novo model of inheritance. 31 / 126 genes show evidence for causation based on gene constraint, chondrocytes expression levels, animal models, short stature associated CNVs, functional GO terms and known protein interactions.

In conclusion, exome analyses of 200 patients with ISS identified a known cause of shorted stature in 11% of cases. Furthermore, we found 126 potential novel candidate genes in 62% of the individuals including 6 genes with independent mutations in two individuals each. Thus, our data strongly suggest that single gene defects may be a frequent cause for idiopathic short stature.

### C24.2

#### Targeted Next Generation Sequencing in skeletal dysplasias: experience on 216 patients

C. Michot<sup>1</sup>, S. Hanein<sup>2</sup>, C. Haudry<sup>3</sup>, G. Bajat<sup>1</sup>, S. Monnot<sup>2</sup>, A. Munnich<sup>1</sup>, J. Bonnefont<sup>3</sup>, V. Cormier-Daire<sup>1</sup>, <sup>1</sup>Genetics Department, INSERM UMR1163, Paris Descartes-Sorbonne Paris Cité University, Imagine Institute, Necker-Enfants Malades Hospital, Paris, France, <sup>2</sup>Imagine Institute, Necker-Enfants Malades Hospital, Paris, France, <sup>3</sup>Molecular Biology Department, Paris Descartes-Sorbonne Paris Cité University, Necker-Enfants Malades Hospital, Paris, France.

Short stature is a common reason for paediatric consultations. After having excluded digestive, renal, inflammatory, metabolic or endocrinologic aetiologies skeleton X-rays may orientate toward the diagnosis of skeletal dysplasias. Next generation sequencing (NGS), allowing simultaneous analysis of many genes, has improved the ability to perform genetic testings for such heterogeneous related conditions. The study reports the results of the analyses of 216 patient DNAs by targeted NGS.

Medical files and X-rays from all patients included were first reviewed by Centre of Reference for Constitutional Bone Dysplasias. A targeted NGS pa-

nel for skeletal dysplasias was designed to sequence 70 genes. Amplicons were captured by Sure-Select technomogy (Agilent technologies) and sequenced on HiSeq (Illumina). Alignment and detection of variants were realized thanks to Burrows-Wheeler and Genome Analysis Toolkit softwares before annotation and analysis by a homemade web interface (PolyWeb). 216 DNAs have been analyzed. For 61 patients (28 %), a pathogenic variant was identified and confirmed by Sanger sequencing. Many variants of unknown significance (VOUS) were found. In 63 cases (30 %) the VOUS were consistent with the phenotypes; familial segregation studies and cDNA analysis (for some) are still in progress. Finally, analyses were negative in 92 cases (42%).

Targeted NGS is efficient to establish the molecular diagnosis of skeletal dysplasias, especially in subgroups with high genetic heterogeneity, such as epiphyseal dysplasias. The important number of VOUS highlights the importance of clinico-biological staffs. Such expertise further emphasizes the need for Centre of Reference for rare diseases.

### C24.3

#### NUP107 mutations cause autosomal recessive inherited early childhood-onset steroid resistant Nephrotic syndrome

N. Miyake<sup>1</sup>, H. Tsukaguchi<sup>2</sup>, E. Koshimizu<sup>1</sup>, A. Shono<sup>3</sup>, N. Matsumoto<sup>1</sup>,

<sup>1</sup>Yokohama City University, Yokohama, Japan, <sup>2</sup>Kansai Medical University, Osaka, Japan,

<sup>3</sup>Kobe University Graduate School of Medicine, Kobe, Japan.

Introduction: Nephrotic syndrome is a renal disorder caused by disruption of the glomerular filtration barrier, resulting in massive proteinuria, hypoalbuminemia, and dyslipidemia. At least 27 genes have been reported to be associated with steroid resistant Nephrotic syndrome (SRNS), but approximately 70% of the patients with childhood-onset SRNS are genetically unknown.

Materials and Methods: To identify the novel responsible gene for SRNS, we performed WES for 18 families in whom the causative mutations have not been identified in the known SRNS genes.

Results: We identified biallelic NUP107 mutations in nine affected individuals from five unrelated families who showed early-onset SRNS. Seven of nine affected individuals had compound heterozygous mutation of one truncating mutation (c.1079\_1083del or c.969+1G>A) and commonly sheared missense mutation (c.2492A>C). They developed nephrotic syndrome from 2 to 3 years of age and progressed to end stage renal disease until 10 years old. The rest of two patients in one family had compound heterozygous mutation of two missense mutation (c.469G>T and c.2492A>C) and showed milder phenotype with later onset (10 to 11 years old). NUP107 encodes Nucleoporin 107kDa (NUP107) is a component of the nuclear pore complex embedded in the nuclear envelope, and ubiquitously expressed including in glomerular podocytes. NUP107 knockdown zebrafish generated by morpholino oligonucleotides displayed hypoplastic glomerulus structures and abnormal podocyte foot processes, which could recapitulate the renal changes in the patients with NUP107 mutations

Conclusions: Here, we identified biallelic NUP107 mutations caused early childhood-onset SRNS and highlighted the importance of nuclear pore complex in human renal disease.

### C24.4

#### Whole-exome sequencing identifies mutations of TBC1D1 encoding a Rab-GTPase-activating protein in patients with congenital anomalies of the kidneys and urinary tract (CAKUT)

A. Kosfeld<sup>1</sup>, M. Kreuzer<sup>2</sup>, C. Daniel<sup>3</sup>, F. Brandt<sup>4</sup>, A. Schäfer<sup>4</sup>, A. Chadt<sup>5</sup>, A. Weiss<sup>4</sup>, V. Riehmer<sup>4</sup>, C. Jeanpierre<sup>6</sup>, M. Klintschar<sup>7</sup>, J. Bräsen<sup>8</sup>, K. Amann<sup>3</sup>, L. Pape<sup>2</sup>, A. Kispert<sup>4</sup>, H. Al-Hasani<sup>5</sup>, D. Haffner<sup>2</sup>, R. G. Weber<sup>1</sup>, <sup>1</sup>Hannover Medical School, Department of Human Genetics, Hannover, Germany,

<sup>2</sup>Hannover Medical School, Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover, Germany, <sup>3</sup>Friedrich-Alexander University of Erlangen-Nürnberg, Department of Nephropathology, Erlangen, Germany, <sup>4</sup>Hannover Medical School, Institute of Molecular Biology, Hannover, Germany, <sup>5</sup>Leibniz Center for Diabetes Research at the Heinrich-Heine- University Düsseldorf, German Diabetes Center, Düsseldorf, Germany, <sup>6</sup>Institut National de la Santé et de la Recherche Médicale UMR1163, Hôpital Necker-Enfants Malades; Université Paris Descartes - Sorbonne Paris Cité, Institut Imagine, Paris, France, <sup>7</sup>Hannover Medical School, Department of Legal Medicine, Hannover, Germany, <sup>8</sup>Hannover Medical School, Department of Pathology, Hannover, Germany.

Congenital anomalies of the kidneys and urinary tract (CAKUT) are genetically highly heterogeneous leaving most cases unclear after mutational analysis of the around 30 causative genes known so far. Assuming that phenotypes frequently showing dominant inheritance, such as CAKUT, can be caused by de novo mutations, de novo analysis of whole exome sequencing data was done on two patient-parent-trios to identify novel CAKUT genes. In

one case, we detected a heterozygous de novo frameshift variant in TBC1D1 encoding a Rab-GTPase-activating protein regulating glucose transporter GLUT4 translocation. Sequence analysis of 100 further CAKUT cases yielded three novel or rare inherited heterozygous TBC1D1 missense variants predicted to be pathogenic. TBC1D1 mutations affected Ser237-phosphorylation or protein stability and thereby act as hypomorphs. *Tbc1d1* showed widespread expression in the developing murine urogenital system. A mild CAKUT spectrum phenotype, including anomalies observed in patients carrying TBC1D1 mutations, was found in kidneys of some *Tbc1d1*-/- mice. Significantly reduced Glut4 levels were detected in kidneys of *Tbc1d1*-/- mice and the dysplastic kidney of a TBC1D1 mutation carrier versus controls. TBC1D1 and SLC2A4 encoding GLUT4 were highly expressed in human fetal kidney. The patient with the truncating TBC1D1 mutation showed evidence for insulin resistance. These data demonstrate heterozygous deactivating TBC1D1 mutations in CAKUT patients with a similar renal and ureteral phenotype, and provide evidence that TBC1D1 mutations may contribute to CAKUT pathogenesis, possibly via a role in glucose homeostasis.

#### C24.5

#### Mutations in KLHL7, a Dominant Retinitis Pigmentosa - Locus, Cause a Recessive Crisponi/CISS1-like phenotype

*L. Crisponi*<sup>1</sup>, *A. Angius*<sup>1</sup>, *P. Uva*<sup>2</sup>, *I. Buers*<sup>3</sup>, *M. Oppo*<sup>4,5</sup>, *A. Puddu*<sup>4</sup>, *S. Onano*<sup>1,6</sup>, *I. Persico*<sup>1</sup>, *A. Loi*<sup>1</sup>, *L. Marcia*<sup>1,6</sup>, *W. Höhne*<sup>6</sup>, *G. Cuccuru*<sup>2</sup>, *G. Fotia*<sup>2</sup>, *M. Deiana*<sup>1</sup>, *M. Marongiu*<sup>1</sup>, *H. Atalay*<sup>6</sup>, *S. Inan*<sup>7</sup>, *O. El Assy*<sup>8</sup>, *L. Smit*<sup>9</sup>, *I. Okur*<sup>10</sup>, *E. Kılıç*<sup>11</sup>, *G. Zampino*<sup>12</sup>, *G. Crisponi*<sup>1,3</sup>, *F. Rutsch*<sup>3</sup>, <sup>1</sup>*Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Cagliari, Italy, Cagliari, Italy,* <sup>2</sup>*Centre for Advanced Studies, Research and Development in Sardinia (CRS4), Science and Technology Park Polaris, Pula, Italy,* <sup>3</sup>*Department of General Pediatrics, Münster University Children's Hospital, Münster, Germany,* <sup>4</sup>*Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Sassari, Italy,* <sup>5</sup>*Cologne Center for Genomics (CCG), University of Cologne, Cologne, Germany,* <sup>6</sup>*Department of ophthalmology, Gazi University School of Medicine, Ankara, Turkey,* <sup>7</sup>*Ophthalmology Department of Afyon Kocatepe University, Afyon, Turkey,* <sup>8</sup>*Pediatric Department-SCBU, Dibba Hospital, Dibba Al Fujairah, United Arab Emirates,* <sup>9</sup>*HagaZiekenhuis Den Haag, Department of Neurology, Den Haag, Netherlands,* <sup>10</sup>*Department of Pediatric Nutrition and Metabolism, Gazi University Medical School, Ankara, Turkey,* <sup>11</sup>*Pediatric Genetics, Pediatric Hematology Oncology Research & Training Hospital, Ankara, Turkey,* <sup>12</sup>*Istituto di Pediatria, Policlinico "A. Gemelli", Università Cattolica del S. Cuore, Rome, Italy,* <sup>13</sup>*Clinica Sant'Anna, Cagliari, Italy.*

**Introduction:** Crisponi syndrome (CS)/Cold-induced sweating syndrome type 1 (CISS1) is a rare autosomal recessive syndrome characterized by a complex phenotype with high neonatal lethality, hyperthermia and feeding difficulties in the neonatal period, scoliosis and cold induced sweating since early childhood. Mutations in the CRLF1 gene are implicated in CS/CISS1. A subset of CS/CISS1 cases remains yet genetically unexplained after CRLF1 sequencing.

**Materials and Methods:** To identify new gene(s) implicated in the clinical phenotype, we used a whole exome sequencing approach followed by targeted Sanger sequencing.

**Results:** We identified homozygous mutations in the KLHL7 gene in five cases. KLHL7 encodes a BTB-Kelch-related protein involved in the ubiquitination of target proteins for proteasome-mediated degradation. Mono-allelic mutations in KLHL7 have been reported in three cases affected by a late-onset form of autosomal dominant retinitis pigmentosa. A critical evaluation of the retinal phenotype in our probands revealed retinitis pigmentosa, so implying that KLHL7 mutations could show greater severity in recessive than in dominant cases.

**Conclusions:** Although these data further support the pathogenic role of bi-allelic KLHL7 mutations in a CS/CISS1-like phenotype, they do not explain all their clinical manifestations and highlight the high phenotypic heterogeneity associated to mutations in KLHL7. Uncovering whether KLHL7 and CRLF1 converge functionally can have important implications in the search for novel disease-associated genes and pharmacological targets.

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#### C24.6

#### Loss-of-function mutations in ELMO2 impeding RAC1 signaling and cell migration cause intraosseous vascular malformation

*A. Cetinkaya*<sup>1</sup>, *J. R. Xiong*<sup>2</sup>, *I. Vargel*<sup>3</sup>, *K. Kosemehmetoglu*<sup>4</sup>, *I. Canter*<sup>5</sup>, *O. F. Gerdan*<sup>6</sup>, *N. Longo*<sup>7</sup>, *A. AlZahrani*<sup>8</sup>, *E. Z. Taskiran*<sup>1</sup>, *L. D. Botto*<sup>7</sup>, *Z. Gormez*<sup>6</sup>, *E. Uz*<sup>9</sup>, *B. Yuksel*<sup>10</sup>, *S. Ruacan*<sup>11</sup>, *M. S. Sağıroğlu*<sup>6</sup>, *T. Takahashi*<sup>12</sup>, *B. Reversade*<sup>1,2</sup>, *N. A. Akarsu*<sup>1,14</sup>;

<sup>1</sup>*Department of Medical Genetics, Gene Mapping Laboratory, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* <sup>2</sup>*Institute of Medical Biology, Human Genetics and Embryology Laboratory, A\*STAR, Singapore, Singapore,* <sup>3</sup>*Department of Plastic and Reconstructive Surgery, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* <sup>4</sup>*Department of Pathology, Faculty of Medicine, Hacettepe University, Istanbul, Turkey,*

<sup>5</sup>*Department of Plastic and Reconstructive Surgery, Faculty of Medicine, Acibadem University, Istanbul, Turkey,* <sup>6</sup>*Advanced Genomics and Bioinformatics Research Center (JGBAM), BILGEM, TUBITAK, Kocaeli, Turkey,* <sup>7</sup>*Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT, United States,* <sup>8</sup>*Maternity Children Hospital, Makkah, Saudi Arabia,* <sup>9</sup>*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey,* <sup>10</sup>*Genetic Engineering and Biotechnology Institute, TUBITAK Marmara Research Center, Kocaeli, Turkey,* <sup>11</sup>*Department of Pathology, Faculty of Medicine, Koc University, Istanbul, Turkey,* <sup>12</sup>*Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom,* <sup>13</sup>*Institute of Molecular and Cellular Biology, Singapore, Singapore,* <sup>14</sup>*Center for Biobanking and Genomics, Hacettepe University, Ankara, Turkey.*

Primary intraosseous vascular malformations are rare and almost exclusively described in sporadic cases involving bones that ossify intramembranously such as the skull. We previously reported an autosomal recessive form of such a disorder, intraosseous vascular malformation (VMOS) [MIM 606893], characterized by severe progressive life-threatening craniofacial vascular malformations and supraumbilical raphe. Homozygosity mapping and massively parallel sequencing identified mutations in ELMO2, which conveys extracellular signals to cytoskeleton, in five unrelated families with eight affected individuals with VMOS. These include two splice site mutations, a one-nucleotide deletion causing frameshift, and a complex rearrangement deleting the first exon. VMOS lesions had bones with abnormally expanded and malformed blood vessels lacking mature vascular smooth muscle layer. ELMO2 deficient cells had a significant downregulation of the associated partner DOCK1, resulting in deficient activation of RAC1 - a key factor in controlling cell migration. Primary fibroblasts from an individual with VMOS had impaired cell migration, an essential process during angiogenesis, which was rescued by external ELMO2 protein. Comparative phylogenetic analysis points out that Elmo2 first appeared with the divergence of jawed vertebrates from others, and might have acquired new functions in jawed vertebrates. Taken together, our study places ELMO2 in a critical position for maintenance of vascular stability in bone tissue, and sheds new light on the understanding of the regulatory mechanisms underlying angiogenesis in intramembranous ossification.

## POSTERS

### P01 Reproductive Genetics/Prenatal Genetics

#### P01.001

#### Trisomy/Tetrasomy 15q in two prenatal cases

*E. Geán*<sup>1</sup>, *D. Escué*<sup>1</sup>, *M. A. Caamaño*<sup>1</sup>, *A. Morales*<sup>1</sup>, *R. Bohera*<sup>1</sup>, *S. Rubio*<sup>1</sup>, *A. Torrents*<sup>1</sup>, *M. García*<sup>2</sup>, *V. Moreno*<sup>2</sup>, *F. Espicho*<sup>2</sup>, *C. Garrido*<sup>1,2</sup>;

<sup>1</sup>*Reference Laboratory, L'Hospitalet de Llobregat (Barcelona), Spain,* <sup>2</sup>*Hospital de Mérida, Mérida, Spain.*

**Introduction:** Tetrasomy 15q is a well-known clinical entity. Sometimes is found in prenatal study like a SMCs or derivative chromosome.

**Case 1:** Amniotic fluid for a 29-year-old woman was referred to our laboratory for atrio-ventricular dilatation, liver arterio-venous malformation and bilateral hydronephrosis of the fetus. Cytogenetic analysis showed 47,XX, + SAC in 65% of metaphases. ISCA array by Agilent of 60K, and we found an amplification of 15q25.2q26.3 region. It seems to be the origin of SAC. To confirm this hypothesis, we applied centromeric probe of chromosome 15 and telomeric probe of 15qtel by FISH, and the result was an i(15)(q25.2q26.3), without centromeric signal.

47,XX,+mar.ish i(15)(q25.2q26.3)(D15Z1x0,D15S936x2).arr[hg19] 15q25.2q26.3(81.795.278-102.383.473)x3

Parental cytogenetic studies were normal, indicating a de novo origin for the SMC.

**Case 2:** Amniotic fluid for a 27-year-old woman was referred to our laboratory for pleural effusion, ascites and hydrops of the fetus at 32 weeks of gestation. Cytogenetic studies in amniotic fluid showed a derivative of chromosome 7. CGH array confirm a terminal deletion on the short arm of chromosome 7 (with deletion of the genes in this region) and amplification of 15q23q26.3, that behaves double gene dosage of the genes contained in that region. No information about cytogenetic studies of the parents has been facilitated.

46,XX,der(7)t(7;15)(p22.3;q26.3).arr[hg19] 7p22.3(101.528-67.231)x1,15 q23q26.3(68.759.755-102.383.473)x3

**Conclusion:** The increasing use of array CGH in clinical laboratories will provide an efficient method for more comprehensive characterization of SMCs and derivative chromosomes. We discuss clinical utility.

**P01.002****Raising confidence threshold increases the positive predictive value of a SNP-based NIPT for the 22q11.2 microdeletion**

A. Ryan, S. Iyengar, B. Jiorle, Z. Demko;  
Natera Inc, San Carlos, CA, United States.

**AIM:** To examine whether raising the confidence threshold at which the algorithm for a SNP-based NIPT for the 22q11.2 microdeletion makes a positive call would increase the positive predictive value (PPV) without affecting test sensitivity.

**METHODS:** 20,776 NIPT samples received between Feb-Aug, 2014 were previously evaluated for the 22q11.2 deletion using primers targeting 672 SNPs in a 2.9 Mb segment of 22q11.2, and analyzed using a high-risk confidence threshold of 0.90. Follow-up information, including ultrasound findings, were collected for high-risk cases. Here, the algorithm's confidence threshold was raised to 0.95, and PPV recalculated for the entire cohort and for the subset of cases with prior-known directly-associated ultrasound anomalies (high a priori risk) and with no prior-known anomalies (low a priori risk).

**RESULTS:** At the original confidence cut-off, the test had a PPV of 18%, which increased to 42.3% upon reflex-sequencing of high-risk samples at a higher read depth (HDOR). Raising the algorithm's confidence level increased the PPV to 52.4% and reduced the false positive rate from 0.12% to 0.07%, with no loss in test sensitivity. The PPV was 100% for high a priori risk cases, and 20% for low a priori risk cases.

**CONCLUSIONS:** The updated methodology (raised confidence level + reflexing) eliminated 80% of the FP cases originally reported, yielding an improved PPV for this SNP-based NIPT (52.4% vs. 18%). Given the higher than previously-reported incidence of the 22q11.2 microdeletion and the benefits of early intervention, we expect this improved test to be highly beneficial in prenatal testing.

**P01.003****5-methylcytosine and 5-hydroxymethylcytosine patterns in human spermatogenic cells**

A. V. Tikhonov<sup>1,2</sup>, O. A. Efimova<sup>1,2</sup>, A. A. Pendina<sup>1,2</sup>, I. D. Fedorova<sup>2</sup>, S. E. Parfenyev<sup>1</sup>, A. S. Koltsova<sup>1,2</sup>, T. V. Kuznetsova<sup>1,2</sup>, V. S. Baranov<sup>1,2</sup>,

<sup>1</sup>Saint Petersburg State University, St.Petersburg, Russian Federation, <sup>2</sup>D.O.Ott Research Institute of Obstetrics, Gynecology and Reproductology, St.Petersburg, Russian Federation.

We aimed to study 5-hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC) patterns in human spermatogenic cells. Samples were obtained by testicular biopsy of 15 patients diagnosed with azoospermia. Chromosome and nuclei preparations were made by "direct" technique using hypotonic (0.9% sodium citrate) and colchicines treatment and fixation with methanol:acetic acid, 3:1. Using indirect immunofluorescence, we have analyzed the localization of 5hmC and its co-distribution with 5mC in the nuclei and chromosomes from spermatogenic cells. To visualize nuclei and to identify chromosomes, we used QFH/AcD-staining. To determine nucleus ploidy, we used FISH with two centromeric DNA-probes.

5mC was detected in all nuclei from both diploid and haploid cells and in all chromosomes from both mitotic spermatogonia and meiotic spermatocytes. 5hmC showed a different pattern: it was present only in some nuclei and was totally absent in mitotic and meiotic chromosomes. By comparing hydroxymethylation and FISH patterns in 5000 nuclei from each sample, we established that 5hmC was present in 32-52% of diploid nuclei from spermatogonia and Sertoli cells. In contrast, among haploid nuclei from spermatids, only 0.6-1.9% was hydroxymethylated.

Thus, in contrast to 5mC, global 5hmC pattern changes during human spermatogenesis. The presence of 5hmC in a minor set of spermatids suggests active demethylation of their genome. The fate of these hydroxymethylated spermatids during spermiogenesis is to be elucidated in further studies.

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**P01.004****Application of a-CGH for preimplantation genetic testing (PGT) in young women and patients of advanced maternal age**

O. A. Yacuts, J. V. Tsukerman, A. B. Zhabinskaya, A. V. Aliakseyeva, O. L. Tishkevich; Centre of assisted reproduction «Embryo», Minsk, Belarus.

PGT was started in our center in 2012 with FISH diagnostics. In 2015 we began applying array comparative genomic hybridization (a-CGH) for preimplantation screening and diagnostics. Indications for PGT included: advanced maternal age (AMA) (>35), repeated implantation failure (>2), balanced chromosome rearrangements.

Materials and Methods: We conducted 14 cycles, IVF+ICSI. Patients were di-

vided into 2 cohorts: 1) young patients (<35) and 2) AMA (>35). Average age was  $29.5 \pm 1.4$  and  $41.2 \pm 2.2$ , respectively. A-CGH (Bluegnome, Illumina) was used to detect whole chromosome aneuploidies. Trophectoderm biopsy was performed on 5-6 day of embryo development. Blastocysts were vitrified and transferred in next cycle after PGT.

Results:

PGT was performed for 48 blastocysts and aneuploidy rates were 53.1% for young patients and 100% for AMA group. The reason for high aneuploidy rate in AMA group is age and small number of blastocysts. Complex chromosome abnormalities were more common in AMA group (Table 1). The most frequent aneuploidies were trisomies (37.5%) and monosomies (35.4%). Aneuploidies for X and Y chromosomes were detected in 8.3%.

	Young patients	AMA
Average number of blastocysts per cycle	4	2,7
Euploid blastocysts, %	46,9	0
Blastocysts with complex chromosome abnormalities, %	15,6	31,2
Failed amplification, %		4,2

Conclusion: In cohort of AMA the average number of blastocysts on day 5 and 6 was significantly lower than in patients of younger age. Complex chromosomal aberrations were double higher in this group.

**P01.005****Male age is not related with high rates of spermatozoa and embryos aneuploidy**

J. A. Ortiz<sup>1</sup>, B. Lledo<sup>1</sup>, R. Morales<sup>1</sup>, A. M. Fabregat<sup>1</sup>, E. Garcia-Hernandez<sup>1</sup>, A. Rodriguez-Arnedo<sup>2</sup>, J. Llacer<sup>2</sup>, R. Bernabeu<sup>2,1</sup>;

<sup>1</sup>IB Biotech, Alicante, Spain, <sup>2</sup>Instituto Bernabeu, Alicante, Spain.

**Introduction:** The frequency of IVF embryo aneuploidies is estimated to be high and its occurrence is related to maternal and paternal factors. The main factor is female age, however paternal factor could play an important role, mainly in patients with abnormal seminal parameters and chromosomal segregation.

**Materials and Methods:** We performed a retrospective observational study (from January 2013 to December 2015). For PGSv2.0 trophoblast genome was amplified and aCGH performed using Agilent SurePrintG3 8x60K. The association between variables and male age was evaluated by logistic regression and chi-square (SPSSv20.0).

**Results:** The seminal parameters and sperm FISH from 428 patients (from 20 to 53 years old) were evaluated. 31% of the analysed patients had an abnormal sperm FISH. No significant differences were reported for male age and abnormal FISH ( $p=0.166$ ), however patients older than 50y showed a tendency to higher sperm aneuploidies (50.0% vs 30.5%,  $p=0.081$ ). To show the effect on embryo aneuploidy we analysed 155 blastocysts from 51 oocytes from donor cycles where the male partner had a normal sperm FISH in order to evaluate the effect of male age in embryo aneuploidies avoiding the confounding effect of female factor and the abnormal FISH. The embryo aneuploidy rate was 26%. In these cycles no significant difference was reported between male age and embryo aneuploidy rate ( $p=0.787$ ) in oocyte donors.

**Conclusions:** Increased paternal age is not associated with sperm and embryos aneuploidies, therefore aging men do not have increased risk of producing chromosomally abnormal offspring.

**P01.006****Novel mutations in the androgen receptor gene in four 46,XY females with complete androgen insensitivity syndrome**

H. Ilgin-Ruhi<sup>1</sup>, H. G. Karabulut<sup>1</sup>, Z. Siklar<sup>2</sup>, E. Gokpinar<sup>1</sup>, M. Gokoglu<sup>1</sup>, M. Berberoglu<sup>2</sup>, A. Tukun<sup>1</sup>;

<sup>1</sup>Ankara University Medical Faculty, Department of Medical Genetics, Ankara, Turkey,

<sup>2</sup>Ankara University Medical Faculty, Department of Pediatric Endocrinology, Ankara, Turkey.

Androgen Insensitivity Syndrome (AIS) which is an X-linked disease, is one of the major cause of 46,XY disorders of sexual development (DSD). There is variable phenotypic expression of the disease, and therefore it is classified into complete (CAIS), partial (PAIS) and mild (MAIS) AIS. Female appearance is characteristic finding for a complete resistance to androgen in an affected person with a male karyotype. Here, we presented four cases with CAIS. Case 1 is a 28-year-old infertile woman. Cases 2, 3 and 4 were evaluated due to primary amenorrhea, 17, 16 and 15 years old, respectively. Patients' physical examination revealed an apparently normal female phenotype. Routine chromosomal analysis showed a 46,XY karyotype. All of them were SRY (+) by FISH and were negative for Y chromosome microdeletions. Clinical

and laboratory investigation confirmed the patients' diagnosis of CAIS. We performed DNA sequencing analysis of all eight exons and respective exon-intron boundaries of the AR gene. The four novel mutations detected in the AR gene were as follows: p.Y408\* (c.1224T>A), p.Q68\* (c.1314C>T) in exon 1 and p.D691E (c.2073C>A), p.G725R (c.3288G>C) in exon 4. To date, more than 1000 distinct mutations in the gene have been reported. We described two novel nonsense and two novel missense mutations. The identified mutations provides further evidence for the correlation between genotype and phenotype corresponding to AIS. This study adds the number of AR gene mutations identified so far.

Refecence

Mongan NP, et al. Androgen Insensitivity Syndrome. Best Pract Res Clin Endocrinol Metab, 2015; 29: 569-580.

#### P01.007

#### The length of the CAG and GGN repeat stretches in the AR gene exert independent and combinatorial effects on reproductive hormones and sperm parameters

**M. Grigorova<sup>1</sup>, M. Punab<sup>2</sup>, T. Kahre<sup>3</sup>, M. Ivandi<sup>3</sup>, N. Tönisson<sup>3</sup>, O. Poolamets<sup>2</sup>, V. Vihlajave<sup>2</sup>, B. Žilaitienė<sup>4</sup>, J. Erenpreiss<sup>5</sup>, V. Matulevičius<sup>4</sup>, M. Laan<sup>1</sup>:**

<sup>1</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia, <sup>2</sup>Andrology Unit, Tartu University Hospital, Tartu, Estonia, <sup>3</sup>Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia, <sup>4</sup>Lithuanian University of Health Sciences, Medical Academy, Institute of Endocrinology, Kaunas, Lithuania, <sup>5</sup>Andrology Laboratory, Riga Stradiņus University, Riga, Latvia.

**Introduction:** Androgen receptor (AR) is a transcription factor regulating the expression of reproduction-related genes. We analysed independent and combinatorial effects of polymorphic CAG- and GGN-triplets within the AR transactivation domain on reproductive parameters in the Baltic young male cohort.

**Materials and Methods:** Baltic young male cohort (n=974, 20±2.1 yrs, sperm concentration 81.6±73.0 mln/mL); genotyping of CAG and GGN repeats by fragment analysis PCR; linear regression (CAG and GGN repeat lengths as continuous variables), CAG and GGN trichotomised as short, intermediate, and long (i.e., CAG≤21, 22≤CAG≤24, CAG≥25; GGN≤22, GGN=23, GGN≥24), and treated as categorical variables for Wilcoxon rank-sum and Kruskal-Wallis tests; tests for nine groups of the combinations of short (s), intermediate (i), and long (l) CAG and GGN repeats (i.e., ICAG-sGGN=long CAG-short GGN, etc.).

**Results:** The major findings of the study were: (1) Significant difference in serum FSH among the carriers of 'long-CAG' (≥24 repeats; median 2.51 IU/L) and 'short-CAG' variants (≤21 repeats, 2.92 IU/L; p=0.0007); (2) Independent associations of 'short-CAG' (71.6 mill/mL) and 'short-GGN' (68.4 mill/mL) stretches with the highest sperm concentration; (3) The lowest sperm concentration (<51.8 mill/mL) among men with the 'long-CAG' and 'long-GGN' combination; (4) A synergistic effect of 'long-CAG' and 'short-GGN' on reduced total testosterone (18.8-19.1 vrs 25.5-28.6 nmol/L for other variants).

**Conclusions:** Our results demonstrate for the first time that CAG and GGN repeats in the AR gene affect male reproductive parameters in a combinatorial way, and may have pharmacogenetic implications in modulating the outcome of hormonal therapy.

#### P01.008

#### Length of the androgen receptor CAG polymorphic repeat sequence is associated with ovarian response to Elonva-controlled ovarian hyperstimulation

**M. Macek, Sr.<sup>1</sup>, G. Griesinger<sup>2</sup>, J. Chrudimská<sup>1</sup>, T. Lerman<sup>3</sup>, T. Borgbo<sup>4</sup>, H. Klucková<sup>1</sup>, M. Macek Jr.<sup>1</sup>, C. Y. Andersen<sup>4</sup>:**

<sup>1</sup>Department of Biology and Medical Genetics, Charles University, Prague, Czech Republic, <sup>2</sup>Department of Obstetrics and Gynecology, Kinderwunschzentrum, Luebeck, Germany, <sup>3</sup>Department of Obstetrics and Gynecology, Kinderwunschzentrum, Luebeck, Germany, <sup>4</sup>Laboratory of Reproductive Biology, Rigshospitalet, Copenhagen, Denmark.

**Introduction:** Previously we have observed that "long" polymorphic CAG repeat sequence (henceforward CAG-RS; 23-26x) of the androgen receptor gene (AR) are associated with significantly decreased intrafollicular fluid testosterone levels, compared to "medium" (20-22x) and "short" (<20x) CAG-RS, as well as with higher conversion of testosterone into estradiol in small antral follicles (PMID: 26404660). The aim of this study was to assess the influence of the CAG-RS length on Elonva-controlled ovarian hyperstimulation (COH) in assisted reproduction.

**Materials and Methods:** The prevalence of the three categories of CAG-RS "length" was assessed in DNA samples from 48 "low", 191 "intermediate" and 34 "high" responders by Sanger sequencing.

**Results:** A significantly decreased prevalence of "short" CAG-RS was found

in 12.5% of low responders, compared to 30.0% and 32.3% in intermediate and high responders, respectively. High responders had significantly increased proportion of shorter CAG-RS and the difference of CAR-RS between alleles with low and higher CAG-RS repeats on both X chromosomes was significant (p<0.0001) between low and high responders. In addition, high responders with shorter CAG-RS were characterized by an association with FSH/receptor gene polymorphisms -29 A/G and p.Asn680Ser, i.e. by an integrated genotype: AG / AsnAsn.

**Conclusion:** Longer CAG-RS are linked to lower response to COH in association with other FSH-R,LH-R and LH polymorphisms, including other genes sensitive to the AR transactivation potential. Supported by 00064203, CZ.2.16/3.1.00/24022 and NF-CZ11-PDP-3-003-2014 to MM.

#### P01.009

#### A possible role of Androgen Receptor CAG/ GGN repeat polymorphisms in therapeutic response of infertile men with Hypogonadotropic Hypogonadism

**A. Mohseni Meybodi<sup>1</sup>, M. Sabbaghian<sup>2</sup>, K. Farahmand<sup>1</sup>, V. Firouzi<sup>1</sup>, Z. Rokhsat Talab<sup>1</sup>, H. HosseiniFar<sup>2</sup>, M. Sadighi Gilan<sup>2</sup>, H. Gourabi<sup>1</sup>:**

<sup>1</sup>Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of, <sup>2</sup>Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of.

**Introduction:** Male hypogonadism is a condition in which testicular function harms and decreased synthesis of sex hormones and incomplete sperm production occurs. Hypogonadotropic hypogonadism (HH) affects the hypothalamus and/or the pituitary gland, so secretion of gonadotropin releasing hormone (GnRH) is absent or insufficient. HH may also result from isolated lack of production or poor biosynthesis of pituitary gonadotropins. Accordingly, these patients required Gonadotropin treatment by means of administration of both FSH and β-HCG to have mature sperms in the ejaculate in order to reach fertility. Besides, testosterone is an androgenic hormone which contributes directly to maintaining and promoting spermatogenesis. Given that the actions of Testosterone are mediated by its interaction with Androgen Receptor (AR), the aim of this study was to evaluate the impact of CAG/GGN three nucleotides repeats expansion in AR gene on the effectiveness of GnRH treatment.

**Materials and Methods:** Sixty-two HH subjects were subdivided according to their response to GnRH: 31 HH men had positive and 31 HH men had negative response to GnRH treatment. The numbers of CAG/GGN in first exon of AR gene was analyzed using Hot Start PCR-Sequencing technique.

**Results:** The average of CAG repeats was statistically higher in patients who had positive response in comparison with patients who hadn't responded to GnRH (p<0.05), but the incidence of GGN repeats were not statistically different among these groups (p>0.05).

**Conclusion:** The results of this study suggest that in contrast to GGN, the length of AR gene CAG repeats polymorphism might affect the response to GnRH in HH men. Accordingly, it can be used as a predictive biomarker for response to GnRH in HH hormonal therapy.

#### P01.010

#### Clinical implementation of a custom oligonucleotide array-CGH. Experience in a cohort of +2100 prenatal samples.

**C. Hernando<sup>1</sup>, O. Villa<sup>1</sup>, N. Fornés<sup>1</sup>, S. Cano<sup>1</sup>, H. Mattlin<sup>1</sup>, M. Garcia<sup>1</sup>, L. Armengol<sup>1</sup>, L. A. Pérez-Jurado<sup>2</sup>:**

<sup>1</sup>qGenomics Laboratory, Esplugues de Llobregat, Spain, <sup>2</sup>Universitat Pompeu Fabra-Unitat de Genètica, Barcelona, Spain.

**Introduction:** The implementation of genomic array in prenatal diagnosis has demonstrated the ability of this technique to fulfil the longstanding need for a diagnostic test with a higher resolution and higher diagnostic yield than its predecessor, the conventional karyotype.

**Materials and methods:** Array CGH analysis was performed prospectively in +2100 prenatal samples with indication for invasive testing, using a custom 60k oligonucleotide-microarray (qChip CM), enriched in pericentromeric, subtelomeric and disease-associated genomic regions, in order to maximize the detection of clinically relevant copy-number alterations and minimize the detection of variants of unknown significance (VOUS). As a general rule, VOUS with no clear phenotypic effect (according to current knowledge) and some susceptibility variants were not reported.

**Results:** The genetic analysis of 2146 prenatal samples from different origins allowed us the identification of 151 pathogenic or probably pathogenic alterations (7.03%) and 57 VOUS (2.65%). As expected, the greatest pathogenic detection rate (71.52%, 108/151) was found in fetuses with ultrasound abnormalities. Regarding the VOUS detection, almost all of parental analysis (when available) were inherited from a non-affected parent

(88.46%, 23/26).

**Conclusions:** Our series demonstrates the utility of prenatal microarray testing, since it nearly doubles the diagnostic yield of conventional karyotyping (64/151 with CNVs <10Mb), without a significant increase in the frequency of VOUS. Based on this experience we highlight the importance of pre-test and post-test counselling of couples who are offered prenatal array analysis, and also to establish a fluid communication between the laboratory and the clinicians for the discussion of possible findings.

#### P01.011

#### Evaluation of the diagnostic performance of array CGH analysis in a large cohort of prenatal samples in a public health service

*A. B. Lasierra, M. J. Alcaine, M. D. Miramar, A. Rodríguez, S. Izquierdo, J. I. González, D. Lerma;*  
Hospital Universitario Miguel Servet, Zaragoza, Spain.

**Introduction:** The application of array CGH (aCGH) in prenatal diagnostics enables the detection of submicroscopic copy number variants (CNVs) not detected by karyotyping that can be associated with clinically significant outcomes. However, its use in prenatal diagnosis is discussed and controversial yet. We report the yield of prenatal aCGH test in a public clinical setting. **Materials and Methods:** Since 2014, our Genetics Service included aCGH in prenatal genetic diagnosis routine, previous exclusion of common aneuploidy by QF-PCR/FISH and cytogenetic analysis performed concurrently on all samples tested. The criteria to realise aCGH was abnormal ultrasound scan findings (including NT>P99) in the first or second trimester with normal karyotype. A total of 1043 prenatal samples were analysed by conventional methods: 881 amniotic fluid (AF) and 162 chorionic villi samplings (CVS). aCGH was performed in 192 AF and 31 CVS using qChip Pre (60K).

**Results:** Conventional cytogenetics methods revealed chromosomal abnormalities (aneuploidy, translocations, mosaicism...) in 89/881(10.1%) LA and 44/162(27.2%) CVS. By aCGH, clinically significant CNVs were observed in 5/192(2.6%) AF and 2/31(6.5%) CVS and variants of unclear significance (VOUS) in 9/192(4.7%) AF and 3/31(9.7%) CVS.

Clinically significant CNVs					
Case	Age yrs	Weeks	Indication	aCGH	Parents study
1 AF	32	16	Incomplete AV canal, ostium primum atrial septal defect, hyperdense bowel	arr[hg18] 6q25.3q26(157,533,166-162,090,585)x1 dn	Karyotype and FISH aCGH dn
2 AF	28	21.5	Skeletal dysplasia	arr[hg18] 3q29(197,224,754-198,801,500)x1 dn	Karyotype and FISH aCGH dn
3 AF	34	20.0	Long bones <P5, Interventricular communication NT>P99,	arr[hg18] 5q35.3(178,723,585-180,629,412)x1 dn	Karyotype aCGH dn
4 AF	33	14	pathological ductus venosus	arr[hg18] 1q21.1(144,974,142-146,290,831)x1 mat	Karyotype aCGH mat
5 AF	17	26.2	NT>P99, pathological ductus venosus, megacystis, IUGR and cardiomegaly	arr[hg18] 20q13.12q13.2(42,759,013-49,503,589)x1 arr[hg18] 20q13.31q13.33(55,851,218-61,900,827)x3	Pending
6 CVS	18	13	NT>P99 and IUGR	arr[hg18] 7q32.2q33(29,098,029-132,492,196)x1 dn arr[hg18] 2q11.1q37.3(94,691,169-242,717,069)x2,3 Mosaicism ~41.8%	aCGH dn MS-MLPA
7 CVS	36	13.1	Fetal death at 13 weeks		Karyotype

**Conclusions:** The use of aCGH provides a 3.14%(7/223) incremental yield in prenatal cases with abnormal ultrasound scan findings and normal karyotype. However, the frequency of VOUS detected was superior, 5.4%(12/223), which complicates genetic counselling and increases parental anxiety providing doubts about pregnancy outcome.

#### P01.012

#### Effects of partial AZFc microdeletions on micro TESE results in azoospermic infertile men

*M. Zamanian<sup>1</sup>, M. Seddighi Gilani<sup>2</sup>, A. Miraghazdeh<sup>3</sup>, A. Ghaheri<sup>4</sup>;*

<sup>1</sup>Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of, <sup>2</sup>Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of, <sup>3</sup>Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Science and research branch, Islamic Azad University, Islamic Azad Un., Tehran, Iran, Islamic Republic of,

<sup>4</sup>Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran, Islamic Republic of.

**Introduction:** Microdeletions of Yq chromosome are the most frequent molecular genetic etiology for the male infertility which usually spans AZFa, AZFb and AZFc regions. Microdeletions are mostly seen in AZFc region and usually cover genes actively involved in spermatogenesis. Partial AZFc microdeletion may also happen with various spans namely gr/gr, b2/b3 and

b1/b3. It is known that the micro TESE outcomes as the surgical process for sperm retrieval from the testis in infertile azoospermic men can be predetermined based on the type of AZF microdeletion. Present study was aimed to evaluate the effect of partial AZFc microdeletions on micro TESE results. **Materials and Method:** 200 infertile azoospermic men referred to Royan institute were evaluated for the presence of partial AZFc microdeletions before they undergo micro TESE. Partial AZFc microdeletions were examined through multiplex PCR using seven different STS markers.

**Results:** Among 90 patients (45%) with positive micro TESE results 9 (10%) showed partial microdeletion in AZFc region. From 110 (55%) patients with negative micro TESE results 7 (6.3%) had AZC partial micro deletion.

**Conclusion:** Among 200 patients 16 (8%) showed AZFc partial microdeletions including 11 (5.5%) of gr/gr and 5 (2.5%) of b2/b3. Statistical analysis showed no significant difference on micro TESE results between the patients with and without partial AZFc microdeletions.

#### P01.013

#### Identification of Genetic Mutations Among Male Infertility Saudi Patients using Next Generation Sequencing panels

*M. A. Al Balwi<sup>1,2</sup>, A. AlAbdulrahman<sup>2</sup>, M. Aldrees<sup>2</sup>, A. AlAsiri<sup>2</sup>, M. Ballow<sup>2</sup>, F. AlSalman<sup>1</sup>, I. AlAbdulkareem<sup>2</sup>;*

<sup>1</sup>King Abdulaziz Medical City, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia, <sup>2</sup>King Abdulla International Medical Research Center, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia.

Approximately half of male factor infertility cases have no known reasons in which genetic contribute up to 30% of Male infertility cases including chromosomal abnormality and chromosome Y microdeletion. Genetic expansion testing should be considered for men who exhibited non-obstructive spermatogenic failure such as azoospermia or oligospermia. Here, genetic study were conducted on 130 patients whom diagnosed with azoospermia or oligospermia and referred from IVF and Urology clinics at King Abdulaziz Medical City-Riyadh, Ministry of National Guard. Previously, we reported that about 21 patients (16%) of cases revealed presence of chromosomal abnormalities and 3 patients (2.3%) harboring male factor abnormality that associated with Y chromosome microdeletion(DDX3Y, DAZ1, DAZ2, DAZ3, USP9Y genes).

We designed infertility panel of 72 genes been reported or associated with male infertility using Next Generation Sequencing technology approach. Furthermore, about 81.5% (106 patients) of our cases still idiopathic were screened using this designed infertility panels. To date, there are little know or no molecular analysis studies of male infertility in Saudi Arabia. Using PGM next generation sequencing technology; We identify several novel mutations, SNPs and indels and their frequency among our patients. In this study, we correlated patient's clinical presentation with their mutations finding within the 72 male infertility genes.

#### P01.015

#### Severity of diseases in expanded carrier screening tests of commercial providers

*L. Vidmar, M. Volk, B. Peterlin;*

*Clinical Institute of Medical Genetic, UMC Ljubljana, Slovenia, Ljubljana, Slovenia.*

**Introduction:** As recently recommended by European Society of Human Genetics and American College of Medical Genetics, expanded genetic carrier screening (EGCS) should be focused on severe childhood diseases. The aim of our study was to evaluate and compare commercial providers of EGCS in regard to severity of diseases included in their panels.

**Methods:** We selected four providers and retrieved information on their disease panels from their websites on the 1<sup>st</sup> of February 2016. Disease severity was determined using a recently published algorithm.

**Results:** The analyzed panels included from 75 to 255 diseases, while only 29 diseases were screened by all four providers. Overall, providers screened for 303 diseases of which 35% (range:37-40%) classified as profound, 28% (range:29-38%) as severe, 31% (range:19-28%) as moderate and 6% (range:1-12%) as mild. The composition of panels regarding the severity of diseases differed significantly among the providers, mainly due to different frequencies of mild diseases ( $p=0.008$ ). Nevertheless, it should be noted that the algorithm for disease classification had been designed based on medical professionals' opinions on disease severity, which might not necessarily reflect the lay public opinion.

**Conclusions:** Analyzed panels show great diversity in their composition and size. The majority (68-75%) of diseases included in the panels were classified as either profound or severe, while relative frequency of mild diseases differed significantly among the panels analyzed.

## P01.016

### Noninvasive prenatal diagnosis experience in the Cukurova Region of Southern Turkey: detecting paternal mutations of sickle cell anemia and β-thalassemia in cell-free fetal DNA using high resolution melting analysis

E. Dündar Yenilmez, A. Tuli, C. Evrük;

Cukurova University, Adana, Turkey.

**Introduction** This study used a high-resolution melting (HRM) technique to detect paternal mutations for the noninvasive prenatal diagnosis (NIPD) of β-thalassemia and sickle cell anemia (HbS). We also determined the levels of cell-free fetal DNA and total cell-free DNA.

**Materials and Methods** We used the HRM technique for fetal genotyping of paternal mutations in maternal plasma from 32 pregnancies at risk of β-thalassemia and 57 pregnancies at risk of HbS. The DNA levels in maternal plasma were measured using real-time quantitative PCR. Multiples of the median (MoM) values were calculated in women at risk for β-thalassemia or HbS.

**Results** Twenty-two paternal mutations were detected in 89 pregnant women. Although we were successfully able to detect the paternal β-thalassemia mutations, the mutant HbS fetuses could not be distinguished from maternal background in the early weeks of pregnancy. The detection of DYS14 in male fetuses was 100%. The MoM values of women at high risk of having HbS-affected fetuses were higher than those for the other groups.

**Conclusion** High-resolution melting is a useful method for NIPD of β-thalassemias by detecting paternal mutations in the maternal plasma. Cell-free fetal DNA quantification and MoM values were not informative for HbS or β-thalassemias in early pregnancy.

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## P01.017

### Non-invasive prenatal testing for genome-wide detection of fetal copy number variants reveals aneuploidies in patients with repeated cell-free DNA testing failures

S. Bono, F. Fiorentino, F. Pizzuti, A. Polverari, S. Duca, M. Faieta, A. Nuccitelli, M. Sessa, M. Baldi, L. Diana, F. Spinella;  
GENOMA GROUP, Rome, Italy.

A potential issue with the use of cell-free fetal DNA(cfDNA) testing as a universal method of screening for aneuploidies, is the possibility of failure to provide a result. This is relevant to comparisons with conventional screening, which rarely reports test failures.

Data from pregnant women with repeated quality control(QC) failures(i.e., final analysis metrics do not meet internal QC standards) were retrospectively analysed using bioinformatics methods enabling genome-wide detection of chromosomal copy number variations, in order to ascertain if such failures are caused by biological factors.

Of the 11.584 samples analysed, 12(0.1%) did not returned a result because of a quality metrics failure. In 9 of these samples a single trisomy(T) was identified, whereas the remaining 3 samples showed a double trisomy, one of which involving chromosome 21(T2+T21). Pregnancy follow-up at birth revealed five chromosomal abnormalities that have been overlooked: three cases of fetal mosaicism, one case of UPD15, and one case of T21. All the other pregnancies resulted in a normal karyotype, suggesting confined placental mosaicism occurrences. A retrospective analysis of the 11.572 pregnancies with a normal result for common aneuploidies revealed further 22(0.2%) samples with a single trisomy.

These results demonstrate that aneuploidies other than T21, T18, T13 and sex chromosome aneuploidies, might represent a biological cause of repeated QC failures. cfDNA testing for genome-wide detection of fetal chromosomal abnormalities has demonstrated a useful tool to reduce the incidence of test failures and to improve prenatal management, obtaining comprehensive information about the genetic makeup of the fetus.

## P01.018

### Clinical validation of a non-invasive prenatal test for genome-wide detection of fetal chromosomal abnormalities

F. Fiorentino, S. Bono, F. Pizzuti, A. Polverari, S. Duca, A. Nuccitelli, M. Sessa, M. Baldi, L. Diana, F. Spinella;  
Genoma Group, ROME, Italy.

Current non-invasive prenatal testing(NIPT) approaches, based on analysis of cell-free fetal DNA (cfDNA), involve screening only for common fetal aneuploidies. Such level of screening allows identification of ~83% of chromosomal abnormalities detected by karyotyping in a general screening populati-

on. Hence, a significant proportion(~17%) of fetal chromosomal abnormalities are not detectable by current cfDNA testing methods. To overcome these limitations, NIPT should be extended to cover the entire genome.

Here we present the results of the clinical validation of a novel NIPT designed to detect genome-wide fetal chromosomal abnormalities. A large blinded clinical study was performed, including cfDNA testing of maternal plasma, collected from pregnant women at increased risk for fetal chromosomal abnormalities undergoing invasive prenatal diagnosis. Performance was assessed by comparing test results with findings from G-band karyotyping and/or microarray analysis.

Among 1419 samples analyzed, there were 100 trisomy (T)21 samples, 31 T18 samples, 14 T13 samples, 36 samples with sex chromosome aneuploidies(SCAs) and 37 samples that had positive results for a variety of CNV aberrations, including both sub-chromosomal copy number variants(CNVs) and whole chromosome trisomies. All euploid and chromosomally abnormal samples were classified correctly by NIPT. Clinical sensitivity and specificity within this study was determined to be 100% for T21, T18, T13, SCAs and genome-wide CNVs.

This study has demonstrated that genome-wide non-invasive chromosomal assessment can provide sensitive and specific detection of common and other aneuploidies, as well as a wide range of sub-chromosomal abnormalities, analogous to what can be detected by cytogenetic G-band karyotyping following invasive prenatal diagnosis.

## P01.019

### Clinical validation of the NeoBona test, a new paired-end MPSS approach for cfDNA based prenatal screening of common chromosome aneuploidies

V. Cirigliano, E. Ordoñez, L. Rueda, M. Grau, I. Castilla, M. P. Cañadas;  
Labco Diagnostics, Barcelona, Spain.

**Objective:** To assess the performance of the NeoBona test, based on a novel paired-end massive parallel shotgun sequencing (MPSS) approach for prenatal aneuploidy screening using cell-free DNA (cfDNA) analysis in maternal blood.

**Methods:** Retrospective study of 1730 plasma samples collected in the 1st trimester (11-13 weeks) including 66 confirmed trisomies 21, 34 trisomies 18, 13 trisomies 13 and 8 sex chromosome aneuploidies. The NeoBona test was carried out blindly on 1 ml of plasma using paired-end MPSS and a novel bioinformatics approach generating a unique trisomy score for each chromosome, based on fetal fraction, counting statistics, size distribution and sequencing depth.

**Results:** The NeoBona test provided valid results in 98,9% of cases. All trisomies 21 and 13 were detected (100% sensitivity and specificity) as also 33/34 trisomies 18. All 5 cases of 45,X and 3 with 47,XXY were also correctly identified without false positive results. Normal and aneuploid samples could be correctly scored even at low fetal fractions, in some cases below 1%.

**Conclusions:** Paired-end MPSS allowed simultaneous assessment of fetal fraction, size distribution and chromosome counting which, integrated into a new analysis algorithm allowed the NeoBona test to be successful on a high proportion of samples. Setting analysis cutoffs at the new multifactorial Tscore as evaluated in the course of this study, resulted in high specificity, while also eliminating the need of a lower limit for FF at 4%, thus potentially extending the benefits of cfDNA analysis to a larger proportion of pregnancies.

## P01.020

### Development of a high throughput workflow for genotyping CFTR mutations

T. Hartshorne, J. Lang, E. Swartzman, P. Brzoska, D. de Castro, G. Chow, L. Tracy;  
Thermo Fisher Scientific, South San Francisco, CA, United States.

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CF is the most common genetic disease of Caucasians, affecting 1 in 3000 newborns. To facilitate CFTR genotype analysis, we developed a panel of 200 TaqMan™ SNP Genotyping Assays to specific CF-causing mutations and a high throughput CFTR genotyping workflow. CFTR mutations were selected for TaqMan™ assay development from the CFTR1 and CFTR2 databases based on population frequency and predicted functional activity. Mutations include single nucleotide polymorphisms, small insertion deletions, large deletions, repetitive sequences, and triallelic polymorphisms. The diverse nature of the mutations presented challenges to developing assays that used a single chemistry and that could be run together on one platform. TaqMan SNP assays were initially tested on both 384-well and OpenArray™ (3072-well) plates, run on a real-time PCR system, with Coriell cell line gDNA samples carrying CFTR mutations. As CFTR mutations are

rare and control gDNAs are not always available, assays were also tested with synthetic DNA controls representing all three genotypes per variant to ensure robust allelic discrimination. Any assays that underperformed were redesigned to produce sufficiently robust assays. CFTR assays were additionally tested with DNA isolated from blood and buccal cell samples, and assay accuracy and concordance studies were performed. We present here our development of a complete sample-to-data analysis workflow for high throughput CFTR mutation detection, and provide example assay data and analysis methods for difficult targets such as the 5T/7T/9T polymorphism.

## P01.021

### Implementation of NGS methodology in prenatal testing of Cystic Fibrosis: a diagnostic tool upgrading the quality of genetic services.

V. Papanikolaou<sup>1</sup>, V. Maroulis<sup>1</sup>, E. Darman<sup>2</sup>, M. Vergi<sup>3</sup>, V. Georgiou<sup>3</sup>, M. Papadakis<sup>1</sup>;  
<sup>1</sup>EUROGENETICA SA, Athens, Greece, <sup>2</sup>EUROGENETICA SA, Thessaloniki, Greece,  
<sup>3</sup>Woman's Health, Athens, Greece.

**Introduction:** Cystic fibrosis (CF) is a life-threatening genetic disorder associated with mutations within the CFTR [cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)] gene. Although >90% of CFTR mutations have already been identified, the severity of the disease, the absence of an effective treatment, and the great heterogeneity of mutations, raise the need for a more effective molecular characterization of CF mutations, especially in prenatal diagnosis.

**Materials and Methods:** We validated this approach in a cohort of 298 samples, the majority of participants were pregnant women or couples during screening. Sampling is ongoing. Genomic DNA was amplified using the Ion AmpliSeq™ CFTR panel. DNA libraries were pooled, barcoded, and sequenced using an Ion Torrent PGM sequencer.

**Results:** The NGS method helped identifying among known mutations and polymorphisms, 54 rare variants, each found in just 1/298 samples. Among them, 27 are known as pathogenic or causing mild symptoms or CBAVD and 8 are known to be non-pathogenic. We identified 19 new variants, not included in the known CF mutation databases. These variants, of unknown clinical significance include c.164+18G>A, c.164+1457 delT, p.Leu541Pro, p.Tyr577Cys, p.Glu826Lys, p.Asp836Tyr, p.Val1212Phe, p.Gln1476Glu, c.\*325A>G.

**Conclusions:** The next-generation sequencing technology, fast and cost-effective, applied to the analysis of the CFTR gene on a larger scale of samples should allow a better identification of unknown variants and should be proven a useful tool for the update of the existing mutation databases and the improvement of genetic counseling.

## P01.022

### CGH-array and karyotyping analysis. Comparison in selected cases of prenatal diagnosis.

M. Costa<sup>1</sup>, M. Aviles<sup>2</sup>, S. Arévalo<sup>3</sup>, L. Espí<sup>1</sup>, L. Trobo<sup>1</sup>, E. Garcia-Arum<sup>1,4</sup>, E. Tizzano<sup>1,4</sup>, C. Medina<sup>1</sup>;

<sup>1</sup>Department of Clinical and Molecular Genetics and rare Disease Unit. Hospital Vall d'Hebron, Barcelona, Spain, <sup>2</sup>Unitat Diagnòstic Prenatal. Hospital Vall d'Hebron, Barcelona, Spain, <sup>3</sup>Unitat de Medicina Fetal. Hospital Vall d'Hebron, Barcelona, Spain, <sup>4</sup>CIBERER. Hospital Vall d'Hebron, Barcelona, Spain.

CGH-array has had a major impact on genetic diagnosis over the last years. Comparing with conventional karyotyping, CGH-array offers higher resolution and faster results, which makes it the first approach for most of postnatal cases as well as selected prenatal diagnosis referral indications. However, conventional karyotyping should still be considered as a valuable tool to confirm a number of CGH-array pathological results or to elucidate the etiological origin of some pathological findings for accurate genetic assessment.

Our Service in a public tertiary Hospital routinely offers QF-PCR and karyotype to all prenatal invasive tests. From 2012 onward, CGH-array has been progressively introduced to selected cases, such as fetal ultrasound abnormalities and genetic family history.

A total of 534 CGH-array prenatal cases were performed including amniocentesis (n=443) and chorionic (n=91) samples employing a 60 K custom oligonucleotide-based CGH-array (qChip Pre v1.1 Complete), that allows a resolution of 100-125 Kb in regions causing genomic disorders. Results were obtained at an average time of 7 days.

Pathologic results were detected in 40 cases (7.5%) 19 of which (47.5%) were confirmed by karyotype. Three different categories of cytogenetic ascertained abnormalities were defined: those that were surely detected by karyotype (n=10), those that were detected due to the CGH-array pathologic result (n=8) that included unexpected possible inherited translocations, and rare findings (n=1) that was defined as a ring of chromosome 15.

We conclude that although CGH-array is an increasing, powerful analysis for prenatal diagnosis procedures, conventional karyotyping is still useful to ascertain specific chromosomal rearrangements.

## P01.023

### Counseling for variants of uncertain significance (VOUS) in the setting of prenatal diagnosis

A. Bar-Shira<sup>1</sup>, D. Brabbing-Goldstein<sup>2</sup>, I. Kaplan-Ber<sup>1</sup>, D. Barel<sup>1</sup>, H. Kolog<sup>1</sup>, T. Naiman<sup>1</sup>, S. Simchoni<sup>1</sup>, A. Orr-Urtreger<sup>1,3</sup>, Y. Yaron<sup>1,3</sup>, A. Reches<sup>1,2</sup>,

<sup>1</sup>Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, <sup>2</sup>Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, <sup>3</sup>Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

**Introduction:** The increased sensitivity of chromosomal microarray (CMA) technology allows for improved detection of genomic alterations. Consequently CMA is now recommended as a first-tier test for evaluating multiple anomalies in the fetus. This technology has been embraced by low risk pregnant women in order to gain as much information about their fetus as possible. While there is an expected increase in the detection yield, there is also an increased risk of detecting VOUSs.

**Objectives:** We describe our results within the unique setting of prenatal counseling, often complicated by the unknown phenotype and the paucity of information available.

**Results:** A total of 750 prenatal samples were processed using CMA. The indications for prenatal testing included: maternal age, abnormal ultrasound findings, family history and parental request. Clinical implication of detected CNVs was determined based on local guidelines and those described in the literature. Of samples tested, 32 (4%) were pathogenic variants, 6 (0.8%) were designated as likely pathogenic, and 55 (7.3%) were designated as VOUSs. Parental testing was done in some cases to determine whether the finding was inherited or *de novo*. All results were discussed in genetic counseling.

**Conclusion:** Physicians and patients alike crave certainty. However, CMA testing often lead to the detection of VOUS, which may be associated with a variable phenotype, reduced expressivity and incomplete penetrance. In such cases, expectant parents are often confused and perplexed. In our experience, proper genetic counseling may alleviate parental anxiety and avoid undue termination of a clinically unaffected fetus.

## P01.024

### Increased chromosome 16 disomy rates in human spermatozoa and recurrent spontaneous abortions

M. Neusser<sup>1</sup>, N. Rogenhofer<sup>2</sup>, S. Dürl<sup>1</sup>, R. Ochsenkühn<sup>3</sup>, M. Trottmann<sup>4</sup>, V. Jurinovic<sup>5</sup>, O. Steinlein<sup>1</sup>, V. von Schönfeldt<sup>2</sup>, C. J. Thaler<sup>2</sup>, S. Müller<sup>1</sup>,

<sup>1</sup>Institute of Human Genetics, University Hospital, Ludwig-Maximilians-University, Munich, Germany, <sup>2</sup>Division of Gynecological Endocrinology and Reproductive Medicine, Department of Obstetrics and Gynecology, Ludwig-Maximilians-University, Munich, Germany, <sup>3</sup>Center for Reproductive Medicine, Munich, Germany, <sup>4</sup>Department of Urology, Ludwig-Maximilians-University, Munich, Germany, <sup>5</sup>Institute for Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University, Munich, Germany.

**Introduction:** Fetal aneuploidy caused by maternal meiotic chromosome segregation errors is an established cause of pregnancy loss. We investigated whether unexplained recurrent spontaneous abortions (RSA) are also associated with increased rates of aneuploidy in spermatozoa of RSA-partners ("RSA males").

**Materials and Methods:** Sperm samples of eleven "RSA males" were evaluated for elevated diploidy and disomy levels of chromosomes 1-22, X and Y by multicolour "sperm-FISH".

**Results:** Compared with base-line aneuploidy rates in healthy males, significantly elevated mean disomy rates ( $p \leq 0.05$ ) were observed for chromosomes 1, 2 and 16, while chromosomes 3, 7, 8, 14, 17 and X were inconspicuous in all eleven patients. We observed increased disomy rates for at least three chromosomes in more than 60% of our patients, but no significant increase of the overall mean sperm disomy or diploidy rate. Importantly, meiotic errors involving chromosome 16 contributed to increased sperm disomy in 7/11 of our patients.

**Conclusions:** The high relative contribution of maternal disomy 16 to aneuploidy related fetal loss is well established. Our data suggest that among paternal meiotic errors nondisjunction of chromosome 16 might have similar relative influence on fetal aneuploidy. Our findings argue against using the "standard clinical FISH probe set" 13/18/21/X/Y in "RSA males", because aneuploidy of chromosome 1, 2, and 16, and probably also 6, 15 and 21 appear to be prevalent in spermatozoa from this particular group of patients, and chromosome 16 appears to be the most promising biomarker for diagnostic and prognostic objects in RSA patients.

## P01.025

**De novo mutations in autosomal recessive congenital malformation**

H. A. Black<sup>1</sup>, D. Parry<sup>1</sup>, S. S. Atanur<sup>1</sup>, D. Ross<sup>1</sup>, E. Rose<sup>1</sup>, H. Russell<sup>2</sup>, S. Stock<sup>3</sup>, J. Warner<sup>4</sup>, M. Porteous<sup>4</sup>, T. Aitman<sup>1</sup>, M. J. Evans<sup>5</sup>;

<sup>1</sup>Centre for Genomic and Experimental Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>2</sup>NHS Fife Fetal Medicine Unit, Victoria Hospital, Kirkcaldy, United Kingdom, <sup>3</sup>Tommy's Centre for Maternal and Fetal Health, MRC University of Edinburgh Centre for Reproductive Health, Edinburgh, United Kingdom, <sup>4</sup>South East of Scotland Clinical Genetics Service, Western General Hospital, Edinburgh, United Kingdom, <sup>5</sup>Department of Pathology, NHS Lothian, Edinburgh, United Kingdom.

Rare developmental disorders are genetically heterogeneous. Genetic testing following a termination of pregnancy, on account of congenital abnormalities, often fails to make a molecular diagnosis. In such circumstances, the recurrence risk for future pregnancies is based on the phenotype alone. For a suspected autosomal recessive syndrome, the risk to future pregnancies would be reported as 25%, assuming inheritance of one causative allele from each parent. We report here results from trio-based exome sequencing of two unrelated fetuses, each with a suspected recessively-inherited developmental disorder, within a larger series of nine trios. In both fetuses, a combination of a *de novo* loss of function mutation on one parental haplotype and an inherited loss of function variant on the other parental haplotype were identified, respectively, as the cause of the recessively-inherited developmental disorders, Ellis-van Creveld and Fraser syndromes. *De novo* mutation has only rarely been reported as a contributory cause of recessive disease, but it markedly reduces the recurrence risk for future pregnancies. Our results suggest that causative *de novo* mutations may contribute to the cause of autosomal recessive disease more frequently than has been considered hitherto.

## P01.026

**RYR1 related congenital myopathy in two sib fetuses conceived through artificial insemination with donor sperm (AID)**

M. Romagnoli, E. Pompili, F. Palombo, E. Bonora, M. Seri;

Medical Genetics Unit, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy.

RYR1-related congenital myopathies present clinical, histological, and genetic heterogeneity: a subset of patients has an antenatal onset form of the disorder, characterized during pregnancy by decreased fetal movements, polyhydramnios and arthrogryposis.

We present a case of two sib fetuses, affected by antenatal onset myopathy, conceived through artificial insemination with donor sperm. The first sib presented a NT of 7,76 mm at combined first trimester screening test. At 12+0 gestational week, ultrasound examination revealed large nuchal septated cystic hygromas, mild ascitic effusion, absent fetal movements, contracted limbs and club feet. No cardiac or encephalic malformations were detected and biometric parameters were normal. The pregnancy was terminated. Karyotype formula was 46,XY. Testing for myotonic dystrophy (DM1) was negative.

In the second pregnancy, conceived by the same biological parents, first trimester ultrasound examination demonstrated comparable clinical features (cystic hygromas, absent fetal movements and contracted limbs) and the pregnancy was terminated. Karyotype formula was 46,XX. Exome sequencing performed on the second fetus revealed a compound heterozygosity for two novel mutations in RYR1: a frameshift mutation (g.38951153CG>C) and a splice acceptor site mutation (g.39019237G>A). Sanger sequencing confirmed these mutations and the healthy mother resulted heterozygous carrier of g.39019237G>A. Both variants are predicted to be pathogenetic and clinical findings are suggestive of a RYR1-related congenital myopathy. However, co-segregation studies in the first fetus are needed.

This clinical case confirms the great diagnostic power of whole exome sequencing in autosomal recessive disorders and exemplifies the hard challenge of gamete donors' selection process related to ultra-rare diseases.

## P01.027

**Estimation of frequencies of 20 known recurrent pathogenic CNVs in prenatal and postnatal settings**

I. Maya<sup>1</sup>, L. Basel-Vanagaite<sup>1,2,3</sup>, M. Shohat<sup>4</sup>, J. Jeshaya<sup>1</sup>, S. Kahana<sup>1</sup>, T. Tene<sup>5</sup>, L. Cohen Vig<sup>6</sup>, S. Jakobson<sup>1</sup>, I. Fishman<sup>1</sup>;

<sup>1</sup>Raphael Recanati Genetics Institute, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel, <sup>2</sup>The Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,

<sup>3</sup>Felsenstein Medical Research Center, Rabin Medical Center, Petah Tikva, Israel, <sup>4</sup>The Sackler Faculty of Medicine, Tel Aviv University, tEL aVIV, Israel, <sup>5</sup>Genetics Institute, Meir Medical Center, Kfar-Saba, Israel, <sup>6</sup>Schneider Children's Medical Center of Israel, Petah Tikva, Israel.

**Background:** Chromosomal microarray (CMA) testing is widely used in prenatal and postnatal setting. Penetrance of many copy-number variants (CNVs) is incomplete; this poses challenges for genetic counseling. The purpose of our study was to compare the prevalence of 20 known recurrent pathogenic CNVs in prenatal and postnatal cohort.

**Methods:** During 2010-2016 our clinical hospital-based laboratory performed 8500 tests on index low-risk prenatal samples (3600), fetuses with congenital malformations (2750) and postnatal samples (2150). Recurrent pathogenic CNVs were classified as high-penetrance (>40%; 8 CNVs), moderate-penetrance (10-40%; 8 CNVs) and low-penetrance (<10%; 4 CNVs). We calculated the prevalence of these CNVs in: I) normal fetuses, II) fetuses with malformations, III) individuals with developmental disabilities/congenital malformations.

**Results:** CNVs were detected in 2% of prenatal samples (139/6350) and 6% (125/2150) of postnatal samples. Prevalence of high-penetrance CNVs was 0.3% in group I, 0.6% in group II and 2.4% in group III and of moderate-penetrance CNVs 0.5% in group I and II and 1.2% in group III. Prevalence of low-penetrance CNVs was similar in all groups - e.g for 15q11 microdeletion (NIPA1) it was 0.7% in all groups.

**Conclusions:** We show that the prevalence of low-penetrance CNVs is similar in low-risk prenatal and in postnatal cohort, pointing that these CNVs may have a minor contribution to the overall heritability of developmental disorders. Information on prevalence for different pathogenic CNVs, especially as it pertains to diagnostic yield in genetic testing, should be useful to clinicians considering chromosomal microarray analysis in fetuses with and without structural anomalies

## P01.029

**Offspring of a male 45,XY,der(22;22)(q10;q10) carrier**

E. van Erp;

Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

**Introduction:** In about 5% of the couples with multiple miscarriages a balanced chromosomal aberration in one of the partners is detected.

**Case:** In our case, father is carrier of a der(22;22)(q10;q10) and as such can produce gametes with either two chromosomes 22 or none, both leading to a miscarriage due to a trisomy or a monosomy 22, respectively. The couple had had two miscarriages, however, they also had a healthy son who showed a 46,XY karyotype. This might indicate UPD22mat or the presence of a normal 46,XY cell line in the gametes of our patient. **Results:** Paternity testing confirmed that our patient indeed was the father. To look for a mosaicism with a normal cell line, karyotyping of a skin biopsy was performed and showed also in all cells a der(22;22). In buccal cells no normal cell line was found either, .

The question is whether the children have a maternal UPD22. In literature 4 cases have been described of a UPD22, all as a result of a trisomy rescue mechanism and never a monosomy rescue. No cases of multiple children with UPD22 in one family have been reported as yet.

**Conclusion:** Normally we tell carriers of a der(22;22)(q10;q10) that they can not have biological children. Our case shows that this isn't always true. Two explanations therefore could be: there is a normal cell line present in the gametes of the father leading to normal sperm cells, or the healthy children have a maternal UPD22. These studies are ongoing.

## P01.030

**Discordant genotypic sex and concordant phenotypes in two Spanish siblings with 17 $\alpha$ -hydroxylase/17,20-lyase deficiency carrying the most prevalent mutated alleles in Brazilian patients**

M. Fernández-Cancio<sup>1</sup>, L. Audi<sup>1</sup>, E. García-García<sup>2</sup>, I. J. Arnhold<sup>3</sup>, B. B. Mendonça<sup>3</sup>, E. García-Arumí<sup>1</sup>, E. F. Tizzano<sup>4</sup>, A. Carrascosa<sup>1</sup>;

<sup>1</sup>Paediatric Endocrinology Unit, Vall d'Hebron Research Institute and Hospital, CIBERER, Autonomous University, Barcelona, Spain, <sup>2</sup>Paediatric Service, Hospital Virgen del Rocío, Sevilla, Spain, <sup>3</sup>Division of Endocrinology, Hospital das Clínicas of the University of São Paulo, São Paulo, Brazil, <sup>4</sup>Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron and CIBERER, Barcelona, Spain.

**Introduction:** 17 $\alpha$ -hydroxylase/17,20-lyase deficiency is a rare form of congenital adrenal hyperplasia caused by mutations in *CYP17A1*, characterized by reduction of androgens, estrogens and cortisol production and mineralocorticoid excess.

**Patients and Methods:** Two sisters, phenotypic females, aged 17 and 15, the oldest with 46,XY and the youngest with 46,XX karyotypes, presented with primary amenorrhea and absent secondary sexual characteristics; the eldest presented elevated blood pressure. Both had elevated levels of ACTH, gonadotropins, progesterone, corticosterone and deoxycorticosterone, and reduced estradiol, testosterone, androstendione, 17-OH-P, DHEA-S, cortisol, aldosterone and renin activity. Sanger sequencing was performed to detect

variants/mutations in *CYP17A1* gene.

**Results:** Mutational analysis showed that the two sisters were compound heterozygotes for p.Arg362Cys and Trp406Arg mutations, previously described as the most prevalent mutations in Brazilian families of Spanish (p.Trp406Arg) or Portuguese (p.Arg362Cys) descent. The present siblings were born from parents with origins near Sevilla (Spain). The analysis of 8 polymorphisms in *CYP17A1* in this family and in two Brazilian families, homozygote for each of the mutations, suggested that paternal allele with p.Arg362Cys may have a common origin with the Brazilian carriers while the maternal allele p.Trp406Arg did not.

The clinical and biochemical phenotypes agree with a complete lack of combined 17 $\alpha$ -hydroxylase/17,20-lyase activities. Estrogen therapy and hydrocortisone were given in both cases and gonadectomy was performed in the oldest.

**Conclusions:** Mutations in *CYP17A1* should be investigated in patients with mineralocorticoid excess and deficiency of cortisol, androgens and estrogens. Regardless of genotypic sex, patients with combined 17 $\alpha$ -hydroxylase/17,20-lyase deficiency are treated with glucocorticoids and estradiol.

#### P01.031

#### A novel NIPT approach based on the methylation differences between maternal and fetal DNA

*A. Keravnou<sup>1</sup>, E. Papageorgiou<sup>1,2</sup>, E. Kypri<sup>1,2</sup>, K. Tsangaras<sup>2</sup>, A. Achilleos<sup>2</sup>, P. Mina<sup>2</sup>, C.*

*Loizides<sup>1</sup>, M. Ioannides<sup>2</sup>, G. Koumbaris<sup>1,2</sup>, P. C. Patsalis<sup>1,2</sup>*

*<sup>1</sup>The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>NIPD Genetics Ltd, Nicosia, Cyprus.*

**Introduction:** DNA methylation has been shown to influence tissue specific gene expression and differentiation. Taking advantage of tissue-specific methylation patterns, several groups focused their work on the identification of fetal-specific markers based on the methylation differences between maternal and fetal DNA for the development of non-invasive prenatal testing (NIPT) for fetal aneuploidies.

**Materials and Methods:** Initially, MeDIP-NGS was applied to characterize DNA methylation differences between 3 chorionic villus samples (CVS), 3 female peripheral blood (WBF) samples and 2 plasma samples. A subset of differentially methylated regions (DMRs) was confirmed using MeDIP in combination with a targeted enrichment method followed by NGS. Furthermore, the inter-individual methylation variability of the selected DMRs was investigated and the classification efficiency of the method was verified using normal and trisomy-21 spike-in samples. Finally, normal and aneuploid pregnancies (n=32) were used for the detection of fetal aneuploidies (26 normal, 4 T21, 1 T18, 1 T13).

**Results:** Initially, based on specific selection-criteria 331 hypermethylated fetal-specific DMRs were selected and confirmed in 24 samples ( $p < 2 \times 10^{-16}$ ). Twenty-nine CVS and twenty-eight plasma samples were used to assess inter-individual methylation variability. Trisomy-21 spike-in samples were detected with the z-scores increasing as spiked-in percentage increased. Finally, this method exhibited 100% sensitivity and specificity for all normal, trisomy-21 and trisomy-18 cases. The trisomy-13 case was marginally below threshold.

**Conclusions:** Results show that this approach can potentially be used for the enrichment of fetal DNA in maternal plasma and the development of a novel NIPT method for fetal aneuploidies.

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#### P01.032

#### Genetical study of patients with DSD from Ukraine

*L. Livshits<sup>1</sup>, S. Chernushyn<sup>1</sup>, S. Kravchenko<sup>1</sup>, G. Livshits<sup>1</sup>, V. Pampukha<sup>1</sup>, L. Tavokina<sup>2</sup>, A.*

*Brovko<sup>2</sup>*

*<sup>1</sup>Institute of Molecular Biology and Genetics, Kiev, Ukraine, <sup>2</sup>ISIDA-IVF, Kiev, Ukraine.*

Disorders of sexual development (DSD) are a very important clinical issue with its different aspects relating to diagnosis, treatment and sex of rearing. The classification of ambiguous genitalia in patients is difficult because similar or identical phenotypes may have several aetiologies. In one of every 4500 births, the genital appearance is abnormal and it is not possible to decide at first glance the sex of the infant. To study the genetical aspects of DSD we have collected a cohort of 37 patients with different DSD phenotypes. Using GTG-banded chromosome analysis we identified 4 46,XX males and 32 46,XY females. The karyotype of one male was 45,X+mar. After PCR and FISH analysis of SRY gene location there of four 46,XX males were determined as SRY-positive with Xp;Yp translocations and two 46,XY females were SRY-negative. Rest of 46,XY female results of Sanger sequencing reviled that SRY gene was intact. rs279895, rs376062302, rs531364677, rs200423545

were determined in DMRT1 gene and rs915034 in NR5A1 gene. Exon analysis of one 46,XX SRY-negative male and two 46,XY SRY-positive females are in progress. The project "Genetics of Human Disorders of Sexual Development" is funded by Swiss National Science Foundation.

#### P01.033

#### Ten-year trends in prevalence of Down syndrome in a developing country: impact of the maternal age and prenatal screening

*A. Kurtovic-Kozaric<sup>1</sup>, L. Mehinovic<sup>1</sup>, R. Malesevic<sup>2</sup>, S. Mesanovic<sup>3</sup>, M. Stomornjak-Vukadin<sup>1</sup>, M. Mackic-Djurovic<sup>4</sup>, M. Peric<sup>5</sup>, I. Kurtovic-Basic<sup>1</sup>, M. Kozaric<sup>1</sup>*

*<sup>1</sup>Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina,*

*<sup>2</sup>University Clinical Center Banja Luka, Banja Luka, Bosnia and Herzegovina, <sup>3</sup>University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina, <sup>4</sup>Medical Faculty, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

This study examines trends in total and live birth prevalence of trisomy 21 with regard to increasing maternal age and the introduction of prenatal diagnosis in Bosnia and Herzegovina. The postnatal detection of trisomy 21 has been available since 2005 and prenatal detection since 2008. All centers that diagnose DS prenatally and postnatally in the period from January 01, 2005 to December 31, 2015 were included. In this study, 93 fetuses and 329 babies were diagnosed in the 10-year period. On average, each year 33 DS individuals were born and 13 DS fetuses were diagnosed prenatally. The calculated incidence for the live born DS individuals in Bosnia and Herzegovina is 1:999. Between 2005 and 2015, the LB prevalence of T21 was 9.6 per 10000 births (range 6.3-12.3) and the total prevalence of T21 was 19.1 per 10000 births (range 10.8-25.0). The T21 prevalence per 10000 births increases exponentially with the advanced maternal age. Prenatal prevalence per 10000 births is ~1 for mothers younger than 35, but increases exponentially with advanced maternal age (32 for mothers older than 40 years). The most common indications for invasive prenatal testing were obstetric ultrasound screening combined with biochemical serum analysis followed by the advanced maternal age. There was an increase in total T21 prevalence, mainly explained by increasing maternal age. Live birth prevalence remained stable over time even after the introduction of prenatal diagnosis.

#### P01.034

#### Differentially expressed miRNAs between normal and trisomy-21 placenta - their potential involvement in Down syndrome pregnancy pathologies

*I. Svobodová<sup>1</sup>, P. Calda<sup>2</sup>, M. Brestak<sup>3</sup>, M. Korabecna<sup>1</sup>, E. Pazourkova<sup>1</sup>, S. Pospisilova<sup>1</sup>, M. Krkavcová<sup>4</sup>, M. Novotná<sup>2</sup>, A. Horinek<sup>1</sup>*

*<sup>1</sup>General faculty hospital in Prague, Prague 2, Czech Republic, <sup>2</sup>Department of Obstetrics and Gynecology of the First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague 2, Czech Republic, <sup>3</sup>Screening center of Prague, Prague, Czech Republic, <sup>4</sup>GENVIA genetic laboratories, Prague, Czech Republic.*

**Background:** The Down syndrome (DS) etiology is still unknown. However, all data suggest that the mechanism is more complex than previously supposed. Epigenetic mechanisms, including miRNAs gene expression regulation, are one of potential influencing factors. The aim of this study was to compare miRNAs expressions in placentas with normal and trisomic karyotype and to associate differentially expressed miRNAs with concrete biological pathways.

**Materials and Methods:** In the pilot study, 754 miRNAs were profiled in 30 chorionic villi samples (CVS) - 14 normal and 16 DS, using real-time PCR technology and TaqMan Human miRNA Array Cards A and B. Twelve differentially expressed miRNAs between compared groups of samples were validated on 50 independent samples (25 normal and 25 DS) using TaqMan miRNA Assays. The Mann-Whitney test with a cutoff p-value < 0.05 was used for results evaluation. Functional annotation and diseases association of selected miRNAs were performed using several web-based software tools (miRWalk, DAVID, WebGestalt).

**Results:** Eight miRNAs were verified as upregulated in DS placentas; three of these miRNAs are located on chromosome 21 (miR-99a, miR-125b and let-7c). Many biological pathways associated with DS complications, as neurodegenerative diseases, leukemia, transcriptional regulation or apoptosis, were identified to be potentially disrupted. Moreover, miRNAs overexpressed in DS placenta apparently regulates genes crucial for placenta development (GJA1, CDH11, EGF, ERVW-1, ERVFRD-1, LEP or INHA).

**Conclusion:** Our findings suggest possible participation of miRNAs in etiology of Down syndrome including DS placenta ectopic development.

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**P01.035****Polygenic profiles for predicting risk of early menopause***T. Laisk-Podar<sup>1,2</sup>, A. Salumets<sup>1,2</sup>, R. Mägi<sup>3</sup>,*<sup>1</sup>*Women's Clinic, University of Tartu, Tartu, Estonia, <sup>2</sup>Competence Centre on Health Technologies, Tartu, Estonia, <sup>3</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia.*

Introduction. Reproductive aging impacts female fertility and health, and involves a considerable genetic component as evidenced by recent genome-wide association studies (GWAS). Currently, no genetic markers are used for predicting menopausal age. The aim of this study was to evaluate the applicability of polygenic risk scores (PRS) to predict the risk for early menopause (before age 45).

Material and methods. PRS were generated using the publicly available ReproGen consortium menopausal age GWAS meta-analysis summary statistics including data for 2.4 million markers and involving approximately 70,000 women. Correlation between PRS and menopausal age was tested among 3,189 post-menopausal women in the Estonian Biobank. Receiver operating characteristic (ROC) curves were generated to evaluate the predictive value of PRS for discriminating women with early menopause.

Results. Polygenic risk profiles generated using different marker cut-offs were significantly correlated with age at natural menopause ( $p<0.05$ ). The same profiles were a good predictor of early menopause (AUC up to 0.70), outperforming the predictive value of smoking status (AUC=0.55), which is one of the most important lifestyle factors affecting menopausal age. When the extremes of the PRS were compared, up to 9-fold difference in risk for early menopause was observed.

Conclusion. Polygenic risk profiles show considerable discriminative power for detecting women at risk of early menopause and have the potential to become a valuable tool to increase the accuracy of ovarian reserve assessment, leading to more personalized counselling regarding family planning and patient management.

**P01.036****miR-346 and miR-582-3p regulate EG-VEGF-induced cell invasion and migration through repressing matrix metalloproteinases 2 and 9 in trophoblast cells.***M. Su;**Department of Obstetrics and Gynecology, National Cheng-Kung University Hospital, Tainan, Taiwan.*

MicroRNAs (miRNAs) are noncoding small RNAs emerging as posttranscriptional regulators in various biological processes. Several miRNAs are expressed in human gestational tissue and play some role in embryo implantation and placental development. Some miRNAs are identified to be associated with placenta dysfunction and abnormal pregnancy status, such as intrauterine fetal restriction, preeclampsia, ectopic pregnancy and recurrent miscarriages (RM), but the underlying mechanism is not clear. Recently, EG-VEGF was regarded as a critical factor for embryo implantation and placental development; however, no EG-VEGF related micro-RNAs have been published yet. Two microRNAs (miR-346 and miR586-3p) were predicted to target on 3'UTR of EG-VEGF, and were confirmed to regulate EG-VEGF expression by a luciferase system. Moreover, miR-346 and miR-586-3p were shown to alter intracellular calcium influx and suppress cell invasion ability in trophoblastic cells (HTR-8/SV neo and JAR) through repressing the expressions and activities of MMP-2 and MMP-9 using western blotting and gelatin zymography. In conclusion, miR-346 and miR586-3p could regulate the expression of EG-VEGF and trophoblast cell invasion, which may therefore influence several EG-VEGF related pathophysiology of human pregnancy.

**P01.038****Frequency of chromosomal abnormalities in 4539 Polish patients with fertility disorders. A retrospective data analysis***A. Strychalska, E. Martin, A. Podbiol-Palenta, W. Ratińska, E. Budek, A. Czaja, M. Ptasiński, E. Hnatkiewicz, K. Czop, B. Sokolowska, J. Swadźba; Diagnostyka Sp. z o.o., Cracow, Poland.*

Introduction: Fertility disorders have been a growing issue in modern societies. The successful treatment of these conditions depends on a thorough diagnosis, including karyotype assessment. This study is based on 3 years data and evaluates the prevalence of chromosomal abnormalities in 4539 patients with reproductive disorders.

Methodology: The study includes 4539 patients referred to our laboratory between 2013 and 2015 for routine karyotype assessment. Study groups were defined on the basis of three referrals: infertility, IVF failure and miscarriages. Cytogenetic analysis of peripheral blood lymphocytes was performed using GTG-banding technique, according to the standard algorithms.

Results: Out of 4539 patients (51,27% females, 48,73% males) 126 (2,78%)

carried chromosomal aberrations. There were no significant differences found in the prevalence of abnormalities or the types of aberrations between the groups. The most common chromosomal abnormalities were balanced translocations. Additionally no differences were found in the prevalence of aberrations between the genders. The statistical analysis was done using  $\chi^2$ -test ( $p<0,05$ ).

	The results of karyotype assessment in patients with fertility disorders					
	Infertility		IVF failure		Miscarriages	
	3698 patients	264 patients	577 patients	number	%	number
Translocations	40	1,08%	4	1,51%	8	1,39%
Genosomal mosaics	30	0,81%	1	0,38%	7	1,21%
Numerical aberrations of genomes	17	0,46%	0	0,00%	1	0,17%
Inversions	7	0,19%	1	0,38%	0	0,00%
Other	4	0,11%	1	0,38%	0	0,00%
Deletions	3	0,08%	0	0,00%	0	0,00%
Other mosaics	2	0,05%	0	0,00%	0	0,00%

Conclusions: This study shows 3,5-fold times higher frequency of chromosomal abnormalities in patients with fertility disorders in comparison to the general population. Since the exact reason for referral or the gender does not influence the prevalence of aberrations, all patients with reproductive disorders should be referred for karyotype assessment. It must be noted that balanced translocations can not be distinguished with any other currently used diagnostic method.

**P01.039****A non-invasive bioinformatic method for the analysis of fetal aneuploidies in maternal blood by NGS***K. Ibáñez<sup>1</sup>, D. Prieto<sup>2,3</sup>, J. Silla-Castro<sup>1</sup>, V. F. Montaño<sup>4</sup>, E. Vallespín<sup>4,3</sup>, P. Lapunzina<sup>5,3</sup>, E. Mansilla<sup>2,3</sup>, M. Mori<sup>2,3</sup>, R. Rodríguez<sup>6</sup>, Á. Del Pozo<sup>1,3</sup>, F. García-Santiago<sup>2,3</sup>;*<sup>1</sup>*Bioinformatics Section, Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain, <sup>2</sup>Cytogenetics Section, Institute of Medical and Molecular Genetics (INGEMM). Hospital Universitario La Paz, Madrid, Spain, <sup>3</sup>Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Madrid, Spain, <sup>4</sup>Structural and Functional Genomics section, Institute of Medical and Molecular Genetics (INGEMM). Hospital Universitario La Paz, Madrid, Spain, <sup>5</sup>Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain, <sup>6</sup>Department of Gynecology & Obstetrics. Hospital Universitario La Paz, Madrid, Spain.*

Fetal aneuploidies constitute over 80% of chromosomal abnormalities at birth. Currently, the majority are detected using invasive screening tests, involving both risk to the mother and fetus. Recent studies propose non-invasive methods to detect fetal chromosomal aneuploidies in maternal plasma using common NGS technologies; whole genome sequencing or the selection of various chromosomal loci using a proprietary methodology (DANSRTM), not available to the general scientific community. Hence, we here present a novel custom bioinformatic NGS design for non-invasive prenatal testing. Our strategy employs the dosage detection of an unique set of markers along chromosomes 13, 18, 21, and X, compared to a control (chromosome 1). The design also incorporates chromosome Y markers for sexing, and numerous SNPs for fingerprinting and for the determination of the fractional fetal DNA concentration. Approximately 1,500 very reliable markers have been selected, with lengths ranging from 100 to 300 bp. The sequences have been captured using Roche NimbleGen EZ library and have been sequenced on a MiSeq Illumina platform. A total of 16 samples, including trisomies and normal controls have been used in the validation.

Due to an error in the library generation, experimental modifications are still under development, and thus, the performance of the validation has been lower than expected. Nevertheless, the results demonstrate a 67% of sensitivity and 70% of specificity.

In conclusion, our design would provide a sensitive and robust method for the fetal aneuploidies detection in maternal blood, and could decrease as well the number of invasive tests.

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**P01.040****Uniparental Disomy of Chromosome 8 unmasks a homozygous mutation in the Thyreoglobulin gene causing a rare form of fetal goiter***K. Steindl<sup>1</sup>, R. Müller<sup>2</sup>, P. Joset<sup>1</sup>, B. Oneda<sup>1</sup>, M. Zweier<sup>1</sup>, A. Rauch<sup>1</sup>;*<sup>1</sup>*Institute of Medical Genetics, University of Zurich, Zurich-Schlieren, Switzerland,*<sup>2</sup>*Private outpatient clinic, Winterthur, Switzerland.*

A solid mass within the fetal neck on second trimester ultrasound scan is a

rare finding and can be due to either a teratoma or to a goiter. A goiter is characterized by its typical morphological features, i.e. a homogeneous structure symmetric in shape with increased vascularity within the anterior neck. Fetal goiter can be associated with hyperthyroidism, most frequently caused by maternal antibodies, hypothyroidism due to maternal antithyroid treatment (e.g. Graves' disease) or with a defect within the fetal thyroid gland hormone biosynthesis. We report a rare case of fetal goitrous hypothyroidism in a mother without thyroid gland pathology. TSH, fT3 and fT4 concentrations in the amniotic fluid were determined with amniocentesis. Chromosomal SNP-array analysis on fetal DNA detected isodisomy of chromosome 8 with a long stretch of LOH on the whole chromosome 8. Since UPD 8 may unmask recessive mutations we searched for genes causing autosomal recessive congenital hypothyroidism and found two, the TG and TRHR genes mapping on the long arm of chromosome 8q24.22 and 8q23.1, respectively. At this point we opted for NGS of the Mendelome which revealed a homozygous nonsense mutation in the TG-Gene [c.4588C>T]. Our data support the importance of UPD as an underlying mechanism of rare autosomal recessive disorders. Till now not more than 50 different mutations in the TG gene had been described and thyreoglobulin defects due to biallelic mutations have an estimated incidence of 1 in 100.000.

#### P01.041

#### Prenatal screening shows a remarkably high FMR1 premutation prevalence in the Balearic Islands

D. Heine-Suñer, R. Alfaro Arenas, J. Rosell, Group for the study of FXS in the Balearic Islands;  
Hospital Universitari Son Espases, Palma de Mallorca, Spain.

We conducted a pilot screening study in 3731 pregnant and non-pregnant women in childbearing age of the Balearic Islands. The aims of this study were to determine Fragile X syndrome (FXS) CGG repeat alleles and frequencies in our population and to determine if TP-PCR is an adequate method to perform generalized population prenatal screening. The molecular results of the study are: 1) a prevalence of premutation (PM) carriers of 1 in 106 which is the highest described to date in any population; 2) Information on the AGG interruptions and CGG repeat numbers that showed that the most frequent alleles are 10A9A9 (38,4 %), 9A9A9 (15,1 %) and 10A9 (10, 5 %). Furthermore, alleles with 0 AGG interruptions or with a pure (uninterrupted) CGG repeat run larger than 34 (presumably more unstable), were highest among PM alleles (10 % and 100 % respectively) compared to normal alleles (0, 7% and 0, 07 %, respectively). Collected geographic origins of participants make it very unlikely that the high prevalence observed in this study is originated by founder effects. As for the psychosocial aspects of the study, we found a very high voluntary participation, self-reported high level of satisfaction and low levels of added stress. In addition, we detect low knowledge of the FXS (25%) and irrelevancy of the self-reported health data. The high level of acceptance and unexpected high frequency of expanded PM alleles in females in the Balearic Islands makes a very compelling argument for prenatal and/or preconceptional FXS screening.

#### P01.042

#### Evaluating the correlation between AGG interruptions in the FMR1 gene promoter and allele stability in the population that was evaluated in the Institute of Human Genetics at two large hospitals in Israel during the years 2011-2015

L. Waldman Radinsky<sup>1</sup>, E. Manor<sup>1,2</sup>, I. Menashe<sup>1</sup>, R. Sukenik- Halevy<sup>3</sup>, L. Fridman<sup>3</sup>, A. Shtrich- Asor<sup>3</sup>, A. Koifman<sup>1,2</sup>;

<sup>1</sup>Ben-Gurion University of the Negev, Beer- Sheva, Israel, <sup>2</sup>Soroka University Medical Center, Beer- Sheva, Israel, <sup>3</sup>Meir Medical Center, Kfar Saba, Israel.

The promotor region of the FMR1 gene is characterized by various lengths of CGG repeats. AGG triplicate can be found every 9 CGG repeats in average, usually 1-3 in total. Those triplicate suggested to stabilize carriers of 59-199 repeats. It was previously suggested that a reduction of AGG interruptions is correlated to an increased number of CGG repeats and expansion from carrier to mutated state (>200 repeats).

Aim: to determine the correlation between AGG triplets interruption and allele stability in the Israeli population.

Materials and methods: we conducted a retrospective case-control study assessing AGG interruptions within the promotor of FMR1 gene and the correlation of CGG repeats. Results: No correlation was found between maternal AGG interruptions and allele stability ( $p=0.59$ ). A correlation between CGG repeat numbers to allele instability was shown ( $p=0.003$ ). Maternal ethnicity (Ashkenazi versus non- Ashkenazi) does not affect the chance for unstable transmission ( $p=0.285$ ). We showed a correlation between younger maternal age and a less stable transmission ( $p=0.021$ , OR-0.734,

CI (0.564-0.954)). Two cases in which the fetus inherited a mosaic of the allele were found. We also found a woman who was not a pre-mutation carrier and transmitted the allele in an expanded length to her fetus.

Conclusions: No correlation was found between AGG number abnormalities and allele instability in the tested population. Younger women were found at greater risk for unstable transmission to their fetus. A case of non pre-mutation carrier that can transmit a CGG expansion was recorded. This unusual finding requires further evaluation.

#### P01.043

#### Elevation of GATA-6 and StAR, steroidogenesis pathway genes, in prenatally androgenized rats

M. Salehi Jahromi<sup>1</sup>, A. Zadeh-vakili<sup>1</sup>, F. Ramezani Tehrani<sup>2</sup>, M. Norouz-zadeh<sup>2</sup>, M. Zareksh<sup>1</sup>;

<sup>1</sup>Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Tehran, Iran, Islamic Republic of.

Introduction: Hyperandrogenism, a hallmark of polycystic ovarian syndrome, caused by fetal androgen exposure, is suspected to act via altered expressions of related genes. The aim of this study was to evaluate the expression of GAGT-6 and StAR genes, involved in steroidogenesis pathway, in adult female rats prenatally exposed to androgen excess by comparing them in different phases of estrus cycle with non-treated rats.

Materials and Methods: Eight pregnant Wistar rats in the experimental group were treated by subcutaneous injection of 5 mg free testosterone on day 20 of pregnancy, while controls ( $n = 8$ ) received 500 mL of solvent. Adult female off-springs of each mother were divided into four groups based on observation of their vaginal smear. Along with measurement of serum steroidogenic sexual hormones and gonadotropins levels using ELISA, RNAs were extracted from ovarian theca cells and relative expression levels were measured using Cyber-green Real-Time PCR.

Results: Comparing intervention and control group, relative expression of GATA-6 and StAR increased by 2.08 ( $p<0.001$ ) and 1.4 ( $p<0.05$ ) fold, respectively. Despite changes in expression of each gene in different phases, their trend of expression was almost similar, with the maximum change being observed in diestrus.

Conclusions: This investigation on gene expression changes in ovarian theca cells in prenatally androgenized rats demonstrated elevated expression of the genes studied. Further studies on genes from steroidogenesis and other reproduction related pathways are recommended to confirm these findings and to further explore prenatal effects of excess androgens.

#### P01.044

#### A single-tube tetradecaplex panel of highly polymorphic STR markers for preimplantation genetic diagnosis of hemophilia A by combined mutation detection and linkage analysis

S. S. Chong<sup>1,2</sup>, M. Zhao<sup>1</sup>, P. C. Wong<sup>1,2</sup>, F. S. H. Cheah<sup>2</sup>, J. Mathew<sup>2</sup>, M. Chen<sup>1</sup>, A. S. C. Tan<sup>1,2</sup>;

<sup>1</sup>National University of Singapore, Singapore, Singapore, <sup>2</sup>National University Health System, Singapore, Singapore.

Introduction: Preimplantation genetic diagnosis (PGD) to detect the common hemophilia A (HEMA) inversion mutations represents a major technical challenge. Indirect linkage analysis using polymorphic markers is possible, but is dependent on availability of informative markers. We have developed a single-tube tetradecaplex panel of highly polymorphic microsatellite markers for simplified PGD of HEMA by haplotype linkage analysis with/without mutation detection.

Materials and Methods: Twenty-eight markers <1Mb from F8 were selected for initial testing. Compatible markers were optimized into a single-tube panel and genotyped in 135 Chinese females. The panel was also tested on single cell whole genome amplification (WGA) products. PGD was performed on a couple at risk of transmitting a point mutation, consisting of single-tube multiplex-PCR of fully informative markers and the mutation-site amplicon, followed by GeneScan analysis. Minisequencing was performed on an aliquot of PCR product to detect the point mutation.

Results: Thirteen markers were optimized into a single-tube PCR panel together with AMELX/Y. Observed marker heterozygosities ranged from 0.45 to 0.84, and 80% of the studied population were heterozygous for  $\geq 5$  markers, while 95% were heterozygous for at least one marker on either side of F8. In the IVF-PGD case, the point mutation was successfully detected from single embryo blastomeres and perfectly correlated with a specific marker haplotype.

Conclusions: The single-tube tetradecaplex marker panel provides a common platform containing sufficient marker redundancy for general application to potentially any PGD of HEMA, through haplotype linkage analysis either alone or in combination with mutation detection.

## P01.046

### Genetic variation in leptin and leptin receptor genes as a risk factor for idiopathic male infertility

A. Hodžić<sup>1</sup>, M. Ristanović<sup>2</sup>, B. Zorn<sup>3</sup>, C. Tulić<sup>2</sup>, A. Maver<sup>1</sup>, I. Novaković<sup>2</sup>, D. Plaseska-Karanfilska<sup>4</sup>, B. Peterlin<sup>1</sup>;

<sup>1</sup>Clinical Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia, <sup>2</sup>Institute of Human Genetics, Medical Faculty, University of Belgrade, Belgrade, Serbia, <sup>3</sup>Andrology Unit, Reproductive Unit, Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana, Slovenia, <sup>4</sup>Research Centre for Genetic Engineering and Biotechnology, Skopje, Macedonia, The Former Yugoslav Republic of.

**Introduction:** The current data support an important role of leptin system in the pathophysiology of male infertility in animals and humans. The aim of this study was to examine whether genetic variability in leptin and leptin receptor genes influence male infertility.

**Methods:** We performed a case-control study and were searching for an association between polymorphisms of leptin and leptin receptor genes and male infertility. The study group consisted of 317 patients with idiopathic infertility and a control group of 241 fertile men from Slovenia. Four SNPs in leptin gene and four SNPs in leptin receptor gene were chosen and genotyped. Statistically significant SNP was further validated in additional 255 infertile patients and 168 controls from Serbia and Macedonia.

**Results:** In the Slovenian population we found a statistically significant difference in genotype distribution for rs10244329 polymorphism in leptin gene (recessive genotype model, p value =0.048). The trend toward statistically significant difference in genotype distribution for rs10244329 polymorphism was found also in the Serbian and Macedonian populations (p value=0.09).

**Conclusions:** Our data suggest that genetic variability in the leptin gene might be associated with male infertility warranting further confirmation and mechanistic investigations.

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## P01.047

### Incremental yield of Genomic Microarray in Early Growth Restricted Fetuses Over Karyotyping

A. Borrell<sup>1</sup>, M. Grande<sup>1</sup>, J. Sabrià<sup>2</sup>, E. Meler<sup>3</sup>, A. Muñoz<sup>3</sup>, E. Mazarico<sup>2</sup>, L. Rodriguez-Revenga<sup>1</sup>, F. Figueras<sup>1</sup>;

<sup>1</sup>BCNatal. Hospital Clínic Barcelona, Barcelona, Spain, <sup>2</sup>BCNatal. Hospital Sant Joan Déu, Barcelona, Spain, <sup>3</sup>Hospital Universitari Quirón Dexeus, Barcelona, Spain, <sup>4</sup>Centre Diagnòstic Biomèdic. Hospital Clínic Barcelona, Barcelona, Spain.

**Introduction:** Fetal intra-uterine growth restriction, particularly when early (< 24 weeks) and severe (< 3rd percentile on estimated fetal weight) is associated with fetal genetic disease. However, scarce information is available about this association with microdeletion and microduplication syndromes. The aim of this multicenter study is to assess the incremental yield of genomic microarray over karyotyping in early and severe growth restriction.

**Material and Methods:** This multicenter study included 139 consecutive early and severe growth restricted fetuses, studied during a 3-year period (January 2013 - December 2015) in 3 centers of Barcelona. QF-PCR and BAC (Bacterial Artificial Chromosome) array-CGH (CytoChip Focus Constitutional, BlueGnome, Illumina) were performed in DNA extracted from amniotic fluid after amniocentesis. The incremental yield was defined by the rate of fetuses presenting with a pathogenic copy number variant below 10 Mb in normal QF-PCR results, stratified by the presence of fetal malformations.

**Results:** In our series 9 pathogenic copy number variants were found. Among non-malformed growth-restricted fetuses, a 5.7% (95%CI:1.3 to 10.1)(6/16) incremental yield of genomic microarray was observed over karyotyping, while this rate was 9.1% (95% CI: -0.7 to 18.9) (3/33) in malformed fetuses.

**Conclusion:** The use of genomic microarray provides a 5.7% incremental yield in non-malformed restricted fetuses with a normal karyotype, increasing up to 9.1% in malformed fetuses. These results confirmed previous findings of the single large series on growth restricted fetuses (3% and 10% respectively). Our findings support the use of genomic microarray after a normal QF-PCR or/and karyotype.

## P01.048

### An innovative test for non-invasive Kell genotyping on circulating fetal DNA by means of the allelic discrimination of K1 and K2 antigens

F. Cro<sup>1</sup>, C. Lapucci<sup>1</sup>, E. Vicari<sup>1</sup>, G. Salsi<sup>2</sup>, N. Rizzo<sup>2</sup>, A. Farina<sup>2</sup>;

<sup>1</sup>Geneticlab, Novanta Vicentina, Italy, <sup>2</sup>Division of Prenatal Medicine Department of Medicine and Surgery (DIMEC) University of Bologna, Bologna, Italy.

**Introduction:** Blood incompatibility between mother and fetus causes he-

molytic disease of the fetus and the newborn (HDFN), a potentially fatal condition. The blood groups mainly responsible for the most severe form of HDFN are RhD and Kell. Nowadays fetal Kell genotype can only be detected through invasive procedures, increasing the chance of maternal alloimmunization. Detection of fetal Kell genotype in circulating cfDNA in maternal plasma would enable prevention of HDFN avoiding risks associated to invasive procedures. In this study, an innovative test for Kell genotyping was designed and optimized on cfDNA to create a non-invasive prenatal test for prevention of HDFN caused by Kell incompatibility.

**Materials and Methods:** 17 samples: 1 alloimmunized pregnant woman plasma presenting anti-K antibodies, 6 dilution of kell positive DNA in kell negative DNA and 10 control blood samples were collected and analyzed. Real-time PCR was developed for the allelic discrimination of K1 and K2 and Kell genotype determination.

**Results:** The K1/K2 genotype was correctly determined in all control samples. Results on the Kell positive dilution allow to verify the linearity and efficiency of the method. Results on cfDNA from a Kell-negative pregnant woman confirmed the Kell-positive genotype of the fetus. Fetal fraction was determinate using the Kell-positive DNA quantification.

**Conclusions:** An efficient and reliable strategy for Kell genotyping is herein presented. The method was optimized on cfDNA in order to create a non-invasive prenatal test which could be routinely used for the prevention of hemolytic disease of the fetus and the newborn.

## P01.050

### Clinical findings in Koolen-deVries foetuses

F. García Santiago<sup>1,2</sup>, C. Martínez-Payo<sup>3</sup>, E. Mansilla<sup>1,2</sup>, J. Nevado<sup>1,2</sup>, F. Santos<sup>1,2</sup>, M. Sanz de Pedro<sup>1</sup>, S. Pérez<sup>1</sup>, A. Llorente<sup>1</sup>, P. Lapunzina<sup>1,2</sup>;

<sup>1</sup>INGEMM, Hospital Universitario la Paz, Madrid, Spain, <sup>2</sup>CIBERER, Madrid, Spain,

<sup>3</sup>Ginecología Departamento, Hospital Puerta de Hierro, Madrid, Spain.

**Introduction:** prenatal diagnoses of microdeletion syndromes, which don't show ultrasound alterations until the third trimester, are always difficult, especially if we want to correlate phenotype-genotype. The introduction of microarray based technology enabled genome in prenatal diagnosis resulting in the detection of recurrent microdeletions in foetuses. We try to correlation between these ultrasound alterations and Koolen-de Vries syndrome. This syndrome is characterized by developmental delay, hypotonia, facial dysmorphisms, epilepsy, heart defects and kidney/urologic anomalies.

**Materials and Methods:** We present 4 foetuses with 17q21.31 microdeletion. All of them showed anomalies in the third trimester, 3 ventriculomegaly leve-moderate and one of them TN. 4.2 mm at first trimester, V.I. hypereogenicity, severe CIR and disgenesis of corpus callosum at birth. Array-SNPs performed on uncultured amniocytes. Array-SNPs were performed by HIScan Illumina, ES CYTOSNP850k.

**Results:** All of them revealed a 17q21.31 microdeletion.

**Conclusions:** The appearance of minor malformations in prenatal diagnosis, especially in pregnancies over 22 weeks, is a source of uncertainty when it comes to genetic analysis. However, these findings are the only prenatal expression of many genetic syndromes, as many of them do not present major malformations in this period. From our point of view, it is important to evaluate each case individually and make these genetic studies given the impossibility of another form of screening and its consequences in postnatal life. To our knowledge, this kind of malformations should be a high marker of this syndrome.

## P01.051

### Genetic screening for chromosomal abnormalities, Y chromosome microdeletions and copy number variation in infertile male patients living in the Trakya region of Turkey

H. Gurkan, S. Demir Ulusal, H. Tozkar, E. Atlı, E. I. Atlı, D. Eker;

Trakya University, Medical Faculty, Department of Medical Genetics, Edirne, Turkey.

**Introduction:** Male factor plays important role in the infertility of 40-50 % of infertile couples. Genetic factors such as Y microdeletions associated to testicular function, chromosomal abnormalities and copy number variations (CNVs) of genes have been reported to be risk factors or causes of male infertility.

**Materials and Methods:** In this study 141 male patients (mean age : 33,7) included who have been referred to Trakya University Faculty of Medicine Department of Medical Genetics because of infertility between February 2013- November 2015. 93 of patients (66%) were azoospermic, 48 (34 %) of patients were severe oligozoospermic. Karyotype analysis (GTG banding with a resolution of 450-500) and Y chromosome microdeletion analysis have been performed for all patients. In addition, CNVs were investigated of 39 azoospermic patients (42%) without any structural or numerical chro-

## mosome abnormalities and Y microdeletion

Results: Y chromosome microdeletion or chromosomal abnormality were not found in the severe oligozoospermic patient group. Numerical chromosome abnormalities were found 20.4% (19/93), when structural chromosome abnormalities have been found 3.2% (3/93) and Y chromosome microdeletions have been found 8.6% (8/93) of azoospermic patients. CNVs have been found in the 7 (18%) out of 39 azoospermic patients investigated for CNVs.

Conclusions: Chromosome abnormality ratios have been reported to be between 1.9-20.86 % in different populations of infertile male patients. Chromosomal abnormality frequency was 15.6% (22/141) in our study. Y microdeletion frequency has been reported between 3.15-20.4% in different studies. One of the CNVs defined in this study was associated with Williams Beuren Syndrome.

## P01.052

### MAMLD1 mutations seem not sufficient to explain a 46,XY DSD

#### phenotype

*N. Camats<sup>1</sup>, M. Fernández-Cancio<sup>1</sup>, L. Audi<sup>1</sup>, P. E. Mullis<sup>2</sup>, C. E. Flück<sup>2</sup>;*

*<sup>1</sup>Vall d'Hebron Institut de Recerca, Barcelona, Spain, <sup>2</sup>University Children's Hospital Bern, Bern, Switzerland.*

Introduction: The MAMLD1 gene (Xp28) is thought to cause disorder of sex development (DSD) in 46,XY patients, mostly presenting with hypospadias, and, recently, gonadal dysgenesis. However, there is some controversy about its role in sex development because some MAMLD1 variants are also detected in normal individuals, several MAMLD1 mutations have wild-type (WT) activity, male Mamld1 knockout mouse have normal genitalia and reproduction; and other species with or without DSD harbor also MAMLD1 variants in the genome. We aimed to analyse MAMLD1 sequence variations in individuals presenting with a wide spectrum of DSD phenotypes.

Patients and Methods: Sanger sequencing was performed in 108 46,XY DSD individuals to detect MAMLD1 gene variations/mutations. Functional experiments were completed in non-steroidogenic and steroidogenic cell lines. We assessed transcriptional activity (on Hes3 and CYP17A1 promoters) and expression of WT and mutant MAMLD1. Besides, we tested the MAMLD1 effect on androgen production by testing the CYP17A1 activity.

Results: We found 9 MAMLD1 mutations (7 novel) in 9/108 46,XY DSD patients. In vitro assays revealed that most MAMLD1 variants acted similarly to the WT. Only the L210X mutation showed loss of function in all tests, while variants L724V and S730S showed a decrease in CYP17A1 promoter activation. We found no effect of either WT or any MAMLD1 variant on CYP17A1 enzyme activity. Also, no difference for MAMLD1 protein expression was found, except for a shorter L210X.

Conclusion: Our data support the notion that MAMLD1 sequence variations may not suffice to explain the DSD phenotype in carriers.

## P01.053

### MiR-335-5p, miR-203a and miR-204-5p expression study in fetal and maternal tissues of women with spontaneous abortion in the first trimester of pregnancy

*E. P. Omelchuk, E. V. Butenko, K. A. Kovalenko, E. V. Mashkina;*

*Southern Federal University, Rostov-on-Don, Russian Federation.*

Introduction. Coordinated work of gene cascades in maternal and fetal cells is crucial for mother-placenta-fetus system formation and functioning. Reasons for up to 60% of spontaneous abortions remain unknown. Micro RNAs act as post-transcriptional gene regulators, and affect gene expression directly in the cells of their origin or take part in intercellular signaling through vesicular transport. In current work, we investigated the expression of three micro RNAs in embryonic, chorionic and decidual tissues after spontaneous abortion compared to normal first trimester pregnancies.

Materials and methods. Bioinformatic study was conducted and miR-335-5p, miR-203a and miR-204-5p were chosen for further investigation. Samples of chorionic, embryonic tissue and decidua were taken after surgical termination of normally progressing pregnancies (n = 14) and spontaneous abortion (n = 10) in 5-9 week of gestation. Micro RNA expression was analyzed using quantitative real-time PCR method. Mir-92a-1-5p was used as a reference gene. All experiments were conducted in triplicates. Data were analyzed using the 2- $\Delta\Delta CT$  method.

Results. The expression of miR-203a-3p was decreased in decidual and embryonic tissues in spontaneous abortion compared to normal pregnancy. Expression of miR-204-5p and miR-335-5p was equal in all studied tissues and in spontaneous abortion compared to normal gestation.

Conclusion. The results demonstrated that low miR-203a-3p expression level in decidual and embryonic tissues could be associated with spontaneous

abortion in first trimester of pregnancy. This study was supported by the federal assignment № 6.98.2014/K from Russian Ministry of Science and Education.

## P01.054

### Integrated analysis of miRNA- and mRNA expression profiles in preeclampsia

*O. Biro, B. Nagy, J. Rigo;*

*Semmelweis University, 1st Dept. of Obstetrics and Gynecology, Budapest, Hungary.*

Background: Preeclampsia is the leading cause of maternal and fetal morbidity and mortality, affecting 3-8% of all pregnancies worldwide. Unfortunately, the pathogenesis of preeclampsia is still not clear. miRNAs are small, non-coding RNA molecules, which negatively regulate gene expression. They are associated with several pathological conditions, including pregnancy complications such as preeclampsia. The aim of our study was to find preeclampsia-related miRNA regulatory mechanisms using bioinformatics approaches.

Methods: We analyzed miRNA (GSE57050) and mRNA (GSE73374) expression datasets, which were created under similar experimental circumstances. Differently expressed miRNAs were identified for the estimation of their inhibitory effect. We integrated miRNA and gene expression profiles with the MAGIA web tool, and created a bipartite network from the significant miRNA-mRNA pairs using the Cytoscape software. Two subnetworks were expanded by protein-protein interactions from the HPRD database. We analyzed the network elements using different bioinformatics tools and through literature research.

Results: We created a network, which consists of 85 nodes and 80 edges signaling the connections between 52 regulated genes and 33 miRNAs. 11 of the genes are preeclampsia-related and 9 of them were targeted by multiple miRNAs. 8 miRNAs are associated with preeclampsia, and 13 miRNAs regulated more than one mRNA. Hsa-mir-210 was the highest degree node in the network and its role in preeclampsia is well-known.

Conclusions: We identified several miRNA-mRNA interactions, which may contribute to the pathogenesis of preeclampsia. Further investigations are needed to validate these mechanisms and to unfold the possibilities of developing potential therapeutic targets.

## P01.055

### Contribution of chromosome rearrangements to the etiology of miscarriages

*A. Soler<sup>1,2,3</sup>, C. Morales<sup>4</sup>, I. Mademont-Soler<sup>5</sup>, E. Margarit<sup>1,2,3</sup>, M. Papiol<sup>1</sup>, A. Borrell<sup>1,2,3</sup>, V. Borobio<sup>1</sup>, A. Sánchez<sup>1,2,3</sup>;*

*<sup>1</sup>Hospital Clínic, Barcelona, Spain, <sup>2</sup>CIBER de enfermedades raras (CIBERER), Barcelona, Spain, <sup>3</sup>IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>4</sup>The PGDlab Reproductive Genetic Medicine, Jabriya, Kuwait, <sup>5</sup>Institut d'Investigació Biomèdica Josep Trueta, Girona, Spain.*

Introduction: Around 10-15% of clinically recognized pregnancies result in miscarriage and about 50% of early pregnancy losses has chromosome abnormalities. Most of them are numerical abnormalities such as autosomal trisomies, polyploidies and monosomy X. However, structural chromosome abnormalities are also found in around 5% of the cases, most of them unbalanced, and identified through karyotyping.

Materials and Methods: We describe the chromosome rearrangements detected in a series of 1119 consecutive first trimester spontaneous miscarriages, obtained through CV sampling before evacuation and semi-direct cytogenetic analysis. When an unexpected rearrangement was found in the CV sample, chromosome analysis of parents was performed.

Results: Cytogenetic result was achieved in 1011 samples, from which 711 showed an abnormal karyotype (70.3%). Thirty-six chromosome rearrangements were detected (5.1% of the abnormal cases), 5 balanced (0.7%) and 31 unbalanced (4.7%). Three of the balanced rearrangements presented with an additional trisomy. Among the unbalanced rearrangements there were 10 reciprocal translocations, 5 were previously unknown: 3 of them turned out to be inherited, 1 de novo and 1 with no information. Two inversions were unexpected and turned out to be inherited. Only 2 out of the 12 Robertsonian translocations were previously known; among the unexpected ones, 8 were de novo, 1 inherited and 1 with no information. Conclusions: Karyotyping allows the detection and interpretation of such unexpected chromosome rearrangements and adds valuable information for the genetic counseling of the couple and their family.

This work was partially supported by grant PI11/01841 from F.I.S. of Spanish Ministerio Sanidad y Consumo.

## P01.056

### Placental chromosomal mosaicism in first trimester spontaneous abortions

A. Sánchez<sup>1,2,3</sup>, C. Morales<sup>4</sup>, I. Mademont-Soler<sup>5</sup>, E. Margarit<sup>1,2,3</sup>, M. Rubio<sup>1</sup>, A. Borrell<sup>1,2,3</sup>, M. Muñoz<sup>1</sup>, A. Soler<sup>1,2,3</sup>;

<sup>1</sup>Hospital Clinic Barcelona, Barcelona, Spain, <sup>2</sup>CIBER de enfermedades raras (CIBERER), Barcelona, Spain, <sup>3</sup>IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>4</sup>ThePGDlab Reproductive Genetic Medicine, Jabriya, Kuwait, <sup>5</sup>Institut d'Investigació Biomèdica Josep Trueta, Girona, Spain.

Around 10-15% of clinically recognized pregnancies result in miscarriage and about 50% of early pregnancy losses have chromosome abnormalities. Chromosomal mosaicism in chorionic villi samplings (CVS) is detected in 1-2% of the cases and can involve different numerical and structural chromosome abnormalities. Depending on the distribution of the abnormal cells lineage among the placental tissues, there are different classes of mosaicism. The purpose of this work is to identify the rate and spectrum of placental chromosomal mosaicism involved in first trimester miscarriages in our population.

We present the placental chromosomal mosaicism found in a series of more than one thousand of arrested pregnancies obtained through chorionic villi sampling before evacuation. The chromosomal analysis was carried out by semi-direct/short-term (STC) and long-term culture (LTC).

Of 1011 CVS samples analysed we obtained results in both cultures in 592 (58.5%). Among them 21 (3.5%) showed discrepant karyotypes. The chromosome abnormality was present in STC in 9 cases (43%) while the karyotype obtained in LTC was normal only in two cases (9.5%). In all cases except four, chromosomal abnormality was numerical, and the structural abnormalities were present only in LTC. In 33% of cases we found discrepant abnormal karyotypes between both cultures.

These data underline the importance of karyotyping both placental layers, also in spontaneous abortions, to obtain a reliable result and a predictive value for fetal involvement and provides clinical elements to offer an accurate genetic counselling.

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## P01.057

### Is mitochondrial DNA content in euploid blastocysts established by next generation sequencing (NGS) on Ion Torrent platform applicable as a predictor of successful implantation?

M. Hrubá<sup>1</sup>, R. Vlčková<sup>1</sup>, M. Pitrova<sup>1</sup>, L. Hradecký<sup>1</sup>, J. Zemanová<sup>1</sup>, P. Nováková<sup>1</sup>, V. Petriková<sup>1</sup>, P. Martinek<sup>2</sup>, B. Wirléitner<sup>2</sup>, M. Schuff<sup>2</sup>, A. Stecher<sup>2</sup>, M. Murtinger<sup>2</sup>;

<sup>1</sup>IVF Centers Prof. Zech - Pilsen, Pilsen, Czech Republic, <sup>2</sup>Biopticka laborator, Pilsen, Czech Republic, <sup>3</sup>IVF Centers Prof. Zech - Bregenz, Bregenz, Austria.

Introduction: Mitochondrial DNA (mtDNA) content in preimplantation embryos, assessed predominantly by qPCR, was recently suggested as a potential novel biomarker for selection of the most competent euploid embryo (Fragouli E et al, 2015; Diez-Juan A et al, 2015). The embryos with lower mtDNA content were reported to show significantly better implantation rates. Most importantly, embryos with mtDNA amount exceeding a certain threshold (0.003% by qPCR, 0.070% of total reads by NGS; Fragouli E et al, 2015) did not implant.

Materials and methods: We reanalyzed 56 trophectoderm samples from euploid embryos (determined previously by aCGH) by NGS using the Ion Torrent platform allowing simultaneous CNV analysis of both nuclear and mitochondrial DNA. 18 embryos were transferred (10 SETs, 4 DETs with known mtDNA level and transfer outcome for both embryos). We analyzed a putative correlation of the implantation outcome and the level of mtDNA. Results: NGS confirmed euploidy and established mtDNA amount in all 56 cases (mean 0.064%, median 0.054%). 33% (6/18) of the transferred embryos implanted, while 67% (12/18) did not. Interestingly, mtDNA amount did not differ between implanted and non-implanted embryos (P 0.285). Moreover, in 2 successfully implanted embryos the suggested 0.070% threshold was substantially exceeded (0.368%, 0.146% respectively).

Conclusions: Although the results obtained by qPCR in two previous studies indicated the application of mtDNA content as a predictor of embryo competence to produce viable pregnancy, the results obtained by NGS using the Ion Torrent platform should be further cautiously studied and verified in a larger cohort of samples.

## P01.058

### Investigating the Role of Mitochondria in the Preimplantation Stages of Human Embryonic Development

R. S. Jawdat<sup>1</sup>, W. Parson<sup>2</sup>, N. Lane<sup>3</sup>, S. Sengupta<sup>1</sup>;

<sup>1</sup>Institute for Women's Health, London, United Kingdom, <sup>2</sup>Institut für Gerichtliche Medizin der Medizinischen Universität Innsbruck (GMI), Innsbruck, Austria, <sup>3</sup>Research

Department of Genetics, Evolution and Environment, UCL, London, United Kingdom.

Introduction: Maternal factors that may lead to abnormal preimplantation development include mitochondria, which are the most abundant organelles in the oocyte. Genes required for mitochondrial function are encoded by both mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA) necessitating the coordination between the two genomes. Mitochondrial-nuclear mismatch has been proposed as a cause of embryonic cell death. Changes in ATP synthesis and metabolic reactions in the mitochondrial electron transport chain (ETC) have been linked to female infertility. This study explores the effect of differences in mtDNA and nDNA haplotypes in genes involved in the ETC by assessing preimplantation embryo development.

Methods: Maternal and paternal mitochondrial DNA was analysed from 11 fertile and 12 infertile couples by Long range PCR and NGS. The selected nuclear genes were sequenced using SureSelect QXT from Agilent. Each sample was assigned to a mitochondrial haplogroup using HaploGrep and confirmed by the EMMA software. PGD cycles from the fertile group were compared for the proportions of both oocytes that fertilised and embryos that developed to blastocyst.

Results: Several mitochondrial haplogroups were identified reflecting the diverse ethnic population in London. Overall more couples from the infertile (4/12) had identical mtDNA haplotypes compared to the fertile group (1/11). Within the fertile group the presence of the T haplogroup was associated with poor blastocyst formation. Analysis of the selected nuclear gene sequences as well as investigation of the interaction of the T haplotype SNPs remains to be investigated before mitochondrial nuclear mismatch can be correlated to embryo development.

## P01.059

### Interdisciplinary clinical workup increases diagnostic yield in increased nuchal translucency

D. Niedrist<sup>1</sup>, J. Wissner<sup>2</sup>, K. Frontzek<sup>3</sup>, H. Budka<sup>3</sup>, N. Rupp<sup>4</sup>, R. Caduff<sup>5</sup>, M. Zweier<sup>1</sup>, B. Oneda<sup>1</sup>, P. Joset<sup>1</sup>, R. Asadollahi<sup>1</sup>, A. Rauch<sup>1</sup>;

<sup>1</sup>Institute of Medical Genetics, University of Zurich, Zurich - Schlieren, Switzerland,

<sup>2</sup>Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland, <sup>3</sup>Institute of Neuropathology, University Hospital Zurich, Zurich, Switzerland, <sup>4</sup>Institute of Pathology, University Hospital Zurich, Zurich, Switzerland.

Increased nuchal translucency is an early sonographic marker in pregnancies for chromosomal abnormalities. In case of a normal karyotype, increased nuchal translucency may be associated with fetal malformations and monogenetic syndromes such as Noonan syndrome.

We now report on a 37-year old healthy woman at 13 weeks of gestation. The nuchal translucency was increased, there was growth retardation and oedema of the skin. The chromosomes were found to be normal (Affymetrix HD 2.65M, resolution of about 100kb). As the sonographic markers became worse, NGS (TruSight™ One Sequencing panel, 4613 Gene, illumina Inc.) was performed, but still did not reveal the underlying cause. At week 22 the couple decided for termination of pregnancy. The pathological examination revealed a male fetus with low birth weight, microcephaly and severe contractions with webs. The fetal brain was premature with agenesis of corpus callosum and hydrocephalus internus.

The result of an interdisciplinary case discussion was the clinical suspicion of Neu-Laxova Syndrome.

Reevaluation of NGS data showed only one deleterious missense mutation in the PHGDH-gene, and reevaluation of microarray data showed a downward pattern of markers, indicating the possibility of a deletion affecting PHGDH. By MLPA the deletion of exon 12 and 3'UTR of the PHGDH-gene was confirmed in the fetus and the mother. The healthy father was carrier of the mutation in the PHGDH-gene.

This case highlights the importance of interdisciplinary clinical discussions. The clinical suspicion may drive the new genetic technologies in finding even rare diagnoses in a time and cost saving way.

## P01.060

### First clinical application of paired-end MPSS for cfDNA based prenatal screening of aneuploidies

V. Cirigliano<sup>1,2</sup>, E. Ordoñez<sup>1,2</sup>, L. Rueda<sup>1,2</sup>, I. Castilla<sup>1</sup>, M. Grau<sup>1</sup>, C. Fuster<sup>2</sup>, M. P. Cañadas<sup>1</sup>;

<sup>1</sup>Labco Diagnostics, Barcelona, Spain, <sup>2</sup>Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

Objective: To evaluate the performance of the NeoBona test, a new paired-end MPSS based assay for cfDNA aneuploidy screening, in an average risk population.

Methods: A total of 2855 consecutive samples were collected from pregnant women above 10w of gestation (102 twins), regardless their risk category. Samples were analysed using the NeoBona test, a new paired-end MPSS ap-

proach allowing simultaneous assessment of fetal fraction, DNA fragment size distribution and chromosome counting statistics and providing a novel Tscore value which quantifies the likelihood for chromosome aneuploidy. Results: Patients choose to only assess risk for autosomal trisomies in 43,2% of cases, screening for XY aneuploidies was requested for 56.8% of samples. Twelve cases were not suitable for analysis, 2843 were tested and results obtained for 2779 (97,8%). Patient redraw following test failure provided valid results in 88% of cases. A total of 71 aneuploidies were detected, 63 autosomal trisomies, 8 involving the sex chromosomes. High-risks results were confirmed by invasive procedures in all but 7 cases of autosomal trisomies and 1 false positive trisomy 21 result was observed (0.03%). Invasive procedures were performed in 5/8 cases of XY chromosomes aneuploidies (3 45,X, 1 47,XXY and 1 47,XXX) and no false positive results were observed. No follow up available on normal pregnancies, the majority of which are still ongoing. Conclusions: The new analysis algorithm of the NeoBona test, including fetal fraction, size distribution, chromosome counting and sequencing depth, proved highly efficient allowing detecting chromosome aneuploidies with extremely low false positive and failure rates.

#### P01.061

#### Non-invasive prenatal testing (NIPT) may detect unexpected diagnosis of CAIS/PAIS

*F. Lalatta<sup>1</sup>, D. Quagliarini<sup>2</sup>, V. Nicotra<sup>3</sup>, S. Salmona<sup>4</sup>, S. Guerner<sup>5</sup>, L. Colombo<sup>6</sup>, C. Giavoli<sup>7</sup>, G. Manzoni<sup>8</sup>, V. Pasterski<sup>9</sup>;*

<sup>1</sup>Clinical genetics Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>2</sup>Obstetrics Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>3</sup>Centro di Riferimento Regionale diagnosi e cura delle malattie genetiche, Università degli Studi di Catania, Catania, Italy, <sup>4</sup>Obstetrics, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>5</sup>Genetic Laboratory Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>6</sup>Department of Neonatology, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>7</sup>Endocrinology and Metabolic Diseases Unit, University of Milan, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>8</sup>Pediatric Urology Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>9</sup>Department of Psychology, University of Cambridge, Cambridge, United Kingdom.

Introduction: From the first trimester of pregnancy, free DNA of fetal origin (cell free fetal DNA, cfDNA) is present in maternal blood and can be recovered non-invasively. NIPT is increasingly utilized to screen for the most common fetal aneuploidies, including those involving sex chromosomes. Here we report a case in which we resolved a discrepancy between the foetal genotype and phenotype detected using NIPT.

Case description: a 32-year-old primigravida was referred to our unit at 20 weeks to further investigate a 46,XY karyotype concurrent with female-typical genitalia as visualized in routine ultrasound. Considering possible complete/partial androgen insensitivity syndrome (PAIS/CAIS), the AR was analysed, identifying a novel mutation. Disclosure of the CAIS diagnosis took place at 22 weeks and included participation of specialists in paediatrics, genetics and a paediatric urology. At 30 weeks, a psychological assessment was carried out for both parents. There was evidence of elevated somatization and anxiety in the mother and both parents showed notable traumatic stress. However, both expressed a degree of relief at going into the birth with knowledge of the diagnosis and requested a "birth plan" allowing for privacy with respect to (public) discussion of the diagnosis. The pregnancy was carried to term and the birth was uncomplicated.

Conclusions: Both parents reported satisfaction with the prenatal diagnostic process and were relieved to have a sense of knowledge and control in planning for the child's birth and clinical management. Nevertheless, notable levels of traumatic stress were observed as they acclimated to the diagnosis.

#### P01.062

#### Discordant results between fetal karyotyping and non-invasive prenatal testing by maternal sex chromosomal mosaicism (45,X and 46,XX)

*S. Jung<sup>1</sup>, K. Lee<sup>2</sup>, K. No<sup>1</sup>, K. Lee<sup>1</sup>;*

<sup>1</sup>Molecular Diagnostics Testing Center, Seegene medical foundation, Seoul, Korea, Republic of, <sup>2</sup>Department of Physics, Kangwon National University, Chuncheon, Korea, Republic of.

Background: Non-invasive prenatal testing (NIPT) is a novel accurate screening technology for early detection of fetal chromosomal aneuploidies, but the test performance of NIPT for sex chromosomal abnormality (SCA) is not as good as it is for trisomy 21, 18, and 13. For the diagnosis of SCA, the maternal or fetal mosaicism, maternal chromosomal abnormalities, and multiple pregnancies have been considered as a confounding factor in NIPT.

Here, we present a false positive fetal SCA (45,X) result caused by maternal sex chromosomal mosaicism.

Case: A 36-year-old primigravida was offered NIPT due to advanced maternal age. The NIPT result was available at 13<sup>+</sup>1 weeks and negative for trisomy 21, 18, and 13. However, Turner syndrome (45,X) was reported in NIPT. She underwent an amniocentesis at 15<sup>+</sup>5 weeks in order to confirm the identification of 45,X obtained through the analysis of NIPT and the result of amniocentesis showed that the fetus was normal (46,XY). For discordant result between fetal karyotyping and NIPT, full karyotyping analysis of her peripheral blood was additionally performed. Among the 178 G-banded karyotypes randomly selected, 173 (97.2%) were normal (46,XX), but 5 (2.8%) were abnormal (45,X), indicating that maternal mosaicism for aneuploidy could result in discordant NIPT results.

Conclusion: Our findings indicated that maternal mosaicism of sex chromosome could cause discordant SCA associated with NIPT. We highly recommend that maternal karyotype should be confirmed for the cases with abnormal results in NIPT.

#### P01.063

#### Implementation of a targeted enrichment method for the detection of microdeletion and microduplication syndromes for NIPT

*M. Neofytou<sup>1</sup>, K. Tsangaras<sup>2</sup>, E. Kypri<sup>1,2</sup>, L. Charalambos<sup>1</sup>, P. Mina<sup>2</sup>, M. Ioannides<sup>2</sup>, A. Achileos<sup>3</sup>, G. Koumaris<sup>1,2</sup>, P. C. Patsalis<sup>1,2</sup>;*

<sup>1</sup>The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>NIPD Genetics LTD, Nicosia, Cyprus.

Introduction: Novel targeted enrichment technologies have been recently developed and applied for the detection of chromosomal aneuploidies. However, only few studies have examined the possibility of applying targeted technologies for the detection of clinically relevant copy number variants (CNVs). The purpose of this study was to develop and validate a targeted enrichment method in combination with NGS to enable the detection of CNVs using cell free DNA from maternal plasma.

Materials and Methods: Synthetic microdeletion and microduplication syndrome samples were created by spiking DNA from affected samples into cfDNA derived from non-pregnant female samples. We created 63 samples simulating 5%, 10% and 20% fetal fraction pregnancies. Enrichment probes spanning the critical region of twelve microdeletion and microduplication syndromes were developed and used to perform targeted enrichment using in-solution hybridization. A statistical analysis pipeline that simultaneously tests for aberrations in any of the syndromes was also developed.

Results: The overall preliminary detection rate ranged from 59% at ff 5% to 95% at ff 20%.

Conclusion: These preliminary results, illustrate the feasibility of this novel targeted technology for the detection of pathological CNVs in cfDNA from maternal plasma.

#### P01.064

#### Prenatal detection of tetrasomy 12p using Non-Invasive Prenatal Testing (NIPT)

*Y. Torres<sup>1</sup>, X. da Silva<sup>1</sup>, J. C. Cigudosa<sup>1,2</sup>, J. Suela<sup>1</sup>;*

<sup>1</sup>NIMGenetics, Madrid, Spain, <sup>2</sup>Centro Nacional de Investigaciones Oncológicas, Madrid, Spain.

INTRODUCTION: Non invasive prenatal testing (NIPT) is a recent prenatal screening procedure that estimates the risk of several fetal cytogenetic aberrations by means of analyzing the circulating cell-free fetal DNA (ccfDNA) present in maternal blood. Although NIPT have been used to mainly detect chromosomal aneuploidies such as trisomy 21, 18 or 13, the implementation of deeper and more robust next generation sequencing (NGS) protocols and algorithms are showing that other chromosomes and genomic regions can be analyzed with a reasonable sensitivity and specificity.

METHODS: Fetal DNA was extracted from plasma obtained in a BCT DNA free tube (Streck). Library preparation and sequencing proceedings were performed according to BGI NIFTY protocol. NIFTY algorithm was used to analyze the risk for fetal trisomies and other aneuploidies. Genomic results were validated with DNA extracted from 16th gestational week amniotic fluid using a custom Agilent 60k array-CGH platform designed for prenatal diagnosis.

RESULTS: NIFTY algorithm informed of a high risk for duplication of the whole short arm of chromosome 12 (dup12p). Array-CGH confirmed the aberration and informed of a tetrasomy 12p, compatible with a Pallister-Kilian syndrome. Further ultrasound scanning confirmed the presence of diaphragmatic hernia and other abnormalities compatible with the syndrome.

CONCLUSION: Although NIPT was initially designed for the estimation of fetal trisomies T21, T18 or T13, whole genome sequencing can successfully

detect other microscopical aberrations such as tetrasomy 12p. Further studies and reports must be done in order to dilucidate the role of this technique in prenatal care

#### P01.065

#### Non Invasive Prenatal Testing by digital counting of fluorescently labeled DNA molecules

F. Dahl;

Vanadis Diagnostics, Sollentuna, Sweden.

Several countries have implemented Non Invasive Prenatal Testing (NIPT) to analyze chromosomal abnormalities in high-risk pregnancies. The majority of these tests are performed using sequencing technologies that provide both superior specificity and sensitivity compared to traditional first trimester screening. However, in order to provide all women with high performance prenatal screening, the NIPT assay cost and complexity need to be dramatically reduced. We present a platform that reduces the cost of the analysis by an order of magnitude making the assay available for high throughput diagnostic laboratories.

Our solution, called Smart NIPT technology, provides a very high assay precision by counting individual fluorescently labeled DNA molecules. The technology uses DNA probes to specifically convert chromosomal targets of interest into DNA circles. These circles are then clonally expanded into discrete fluorescently labeled DNA objects. The DNA objects are immobilized on a transparent nanopore filter and finally imaged and counted through the bottom of the well. By capturing thousands of DNA targets from each chromosome, PCR amplification can be avoided with increased assay precision and reduced contamination risks as a result.

We applied the technology to analyze 183 blinded plasma samples of which 15 were from women pregnant with a trisomy 21 fetus. All positive samples were classified correctly, separated from the normal samples with a minimum of 6.6 standard deviations. No false positives were called.

In conclusion, the Smart NIPT platform, supported by convincing clinical data, is a new solution that holds promise to provide NIPT to all pregnant women.

#### P01.066

#### Non Invasive Prenatal Testing : incidental finding of multiple myeloma.

M. Imbert-Bouteille<sup>1,2</sup>, J. Chiesa<sup>1</sup>, J. Gaillard<sup>1</sup>, V. Dorvaux<sup>3</sup>, L. Altounian<sup>4</sup>, E. Mousty<sup>5</sup>, S. Finge<sup>6</sup>, P. Bourquard<sup>7</sup>, E. Legius<sup>8</sup>, P. Vandenberghe<sup>9</sup>;

<sup>1</sup>Medical Genetics Department, Carêmeau University Hospital Center, Nîmes, France,

<sup>2</sup>Genetics Department, Arnaud de Villeneuve Hospital, CHRU, Montpellier, France,

<sup>3</sup>Haematology Department, Mercy Hospital Center of Metz-Thionville, Metz, France,

<sup>4</sup>Cytogenetics Department, Mercy Hospital Center of Metz-Thionville, Metz, France,

<sup>5</sup>Multidisciplinary Prenatal Diagnosis Department, Carêmeau University Hospital Center, Nîmes, France, <sup>6</sup>Biology and Immunology Department, Carêmeau University Hospital Center, Nîmes, France, <sup>7</sup>Clinical Haematology Department, Carêmeau University Hospital Center, Nîmes, France, <sup>8</sup>Human Genetics Department, Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium.

Nowadays, genome-wide approaches are part of Non Invasive Prenatal Testing (NIPT), analyzing fetal cell-free DNA (cfDNA) through a maternal blood sample. Technical issues are not the keypoint anymore. Ethical questions, about their large-scaled implementation and potential diagnosis of sub-chromosomal imbalances, do occupy a major place. Another critical issue is about unexpected finding on maternal cfDNA, which is screened altogether with fetal cfDNA, far away from the original test aim.

We report the case of Mrs R., a French forty-year-old pregnant woman, without any medical history. She underwent NIPT, prescribed because of maternal age in Luxembourg where she lives, and performed in Belgium.

By massive parallel sequencing of cfDNA, an abnormal genome-wide representation profile was detected with a gain of 1q, 6p, and chromosome 15 and the loss of 13, 14 and 22q. This specific abnormalities association, reminiscent of acquired chromosomal imbalances, indicated a possible Multiple Myeloma (MM). Clinical, biological and radiological assessments confirmed a smouldering lambda-type free light chain MM. Array Comparative Genomic Hybridization and Fluorescent In Situ Hybridization were performed on tumor plasma cells from bone marrow puncture : they revealed the same anomalies as those detected by NIPT.

This case-report reminds that global medical and ethical thinking is highly required to plan safeguards against possible abuses allowed by these new techniques. It shows the potential power of this tool, whose consequences should be anticipated so as to enrich prior information given to patients. The real medical benefit of incidental findings that may arise out needs careful assessment.

#### P01.067

#### Clinical potential of effective non-invasive exclusion of KEL1 positive fetuses in KEL1 negative pregnant women

R. Vodicka<sup>1</sup>, J. Böhmová<sup>1</sup>, M. Lubusky<sup>1</sup>, R. Kratochvílová<sup>1</sup>, E. Krejcíriková<sup>1</sup>, M. Janíková<sup>1</sup>, L. Dusek<sup>2</sup>, R. Vrtel<sup>1</sup>, M. Prochazka<sup>1</sup>;

<sup>1</sup>University Hospital and Palacky University Olomouc, Olomouc, Czech Republic,

<sup>2</sup>Masaryk University Institute of Biostatistics and Analyses, Brno, Czech Republic.

Objective: The clinical importance of assessing the fetal KEL genotype is to exclude "K" positive fetuses (genotype KEL1/KEL2) in "K" alloimmunized pregnant women (genotype KEL2/KEL2). Non-invasive assessment of the fetal KEL genotype is not yet available in the Czech Republic. The aim of this study is to assess the fetal KEL1/KEL2 genotype from cell-free fetal DNA in the plasma of KEL2/KEL2 pregnant women.

Methods: The fetal genotype was assessed by minisequencing (a dilution series including control samples). A total of 138 pregnant women (between the 8th and the 23th gestational week) were tested by minisequencing. The fetal genotype was further verified by analysis of a buccal swab from the newborn.

Results: Minisequencing proved to be a reliable method. In 2.2 % of the examined women (3/138), plasma sample testing failed; 94.8 % of women (128/135) had the KEL2/KEL2 genotype, and a total of 3.1 % fetuses (4/128) had the KEL1/KEL2 genotype. Sensitivity and specificity reached 100 %, p <0.0001.

Conclusion: Minisequencing is a reliable method for the assessment of the fetal KEL1 allele from the plasma of KEL2/KEL2 pregnant women.

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#### P01.068

#### Development of a novel targeted assay for non-invasive prenatal testing of fetal trisomies exhibits near-diagnostic accuracy

G. Koumaris<sup>1,2</sup>, E. Kyripli<sup>2</sup>, K. Tsangaras<sup>1</sup>, A. Achilleos<sup>1</sup>, P. Mina<sup>1</sup>, M. Neofytou<sup>2</sup>, V. Velissariou<sup>2</sup>, G. Christopoulou<sup>1</sup>, I. Kallikas<sup>3</sup>, A. González-Liñán<sup>4</sup>, E. Benusiene<sup>5</sup>, A. Latos-Bielensk<sup>6</sup>, P. Marek<sup>7</sup>, A. Santana<sup>8</sup>, N. Nagy, Márta Szell<sup>9</sup>, P. Laudanski<sup>10</sup>, E. A. Papageorgiou<sup>1,2</sup>, M. Ioannidis<sup>1</sup>, P. C. Patalsis<sup>1,2</sup>;

<sup>1</sup>NIPD Genetics, Nicosia, Cyprus, <sup>2</sup>The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>3</sup>AAK Ultrasound and Fetal Medicine Center, Nicosia, Cyprus, <sup>4</sup>Andros Day Surgery Clinic, Palermo, Italy, <sup>5</sup>Vilnius University, Vilnius, Lithuania, <sup>6</sup>Center for Medical Genetics, Poznan, Poland, <sup>7</sup>University of Medical Sciences, Poznan, Poland,

<sup>8</sup>Childhood Hospital Materno-Infantil, Las Palmas GC, Spain, <sup>9</sup>University of Szeged, Szeged, Hungary, <sup>10</sup>Medical University of Bialystok, Bialystok, Poland.

Introduction: There is a great need for the development of highly accurate, cost effective technologies, which can facilitate the widespread adoption of Non Invasive Prenatal Testing (NIPT). We hereby present a novel cost effective assay of unparalleled accuracy which overcomes the limitations of current technologies.

Materials and Methods: This method enables the targeted analysis of selected genomic regions at very high sequencing depth and allows highly accurate fetal fraction determination to ensure extremely accurate aneuploidy detection. The analytical performance of the assay was evaluated in a blind study, which comprised 631 samples derived from pregnancies of at least 10 weeks of gestation that had also undergone invasive testing.

Results: The blind study exhibited 100% sensitivity and specificity and correctly classified 52/52 (95% CI: 93.2-100%) cases of trisomy 21, 16/16 (95% CI: 79.4-100%) cases of trisomy 18, 5/5 (95% CI: 47.8-100%) cases of trisomy 13, and 538/538 (95% CI: 99.3-100%) normal cases. The test also correctly identified fetal sex in all cases (95% CI: 99.4-100%). One sample failed pre-specified assay quality control criteria, and 19 samples were non-reportable due to low fetal fraction.

Conclusions: The clinical impact of free fetal DNA (ffDNA) testing has been significant as indicated by its quick adoption in prenatal care. Our novel technology overcomes limitations of current technologies and exhibits near diagnostic performance. We believe that this method enables accurate and cost-effective non-invasive fetal aneuploidy detection of trisomy 21, 18 and 13, which is critical for wide-spread adoption of NIPT.

#### P01.069

#### Non-invasive prenatal testing or microarray? Additional benefit of microarray analysis in prenatal diagnostic of fetuses with increased risk of common aneuploidies

Z. Vlachová, J. Graakjaer, D. Svaneby, A. Bojesen; Lillebaelt Hospital, Vejle Hospital, Vejle, Denmark.

Introduction: Microarray analysis enables detection of copy number variations, aneuploidies and unbalanced translocations. In prenatal diagnostic, microarray analysis is considered most beneficial in pregnancies with ultrasound abnormalities. We report chromosomal abnormalities detected

by single-nucleotide polymorphism (SNP) array in fetuses with increased risk of common aneuploidies without ultrasound abnormalities. We focus on chromosomal abnormalities that would not be detected by non-invasive prenatal testing (NIPT).

**Materials and Methods:** We performed SNP-array analysis in 358 pregnant women, who underwent chorionic villus sampling or amniocentesis between July 2013 and January 2016. The high risk (HR) group (n=165) included fetuses with abnormal ultrasound findings as defined by Danish Society of Fetal Medicine. The group with increased risk (IR) (n=193) consisted of fetuses without ultrasound abnormalities with risk assessment for common aneuploidies (Astraia) higher than 1/300. SNP-array analyses were performed by using Illumina CytoSNP-12 version 2.1.

**Results:** Abnormal SNP-array results were more prevalent in the HR group 14.5% (n=24) compared with IR group 5.7% (n=11) (p-value 0.007). Within the IR group, nine cases were evaluated as pathogenic. Four of the nine pathogenic chromosomal abnormalities detected by SNP-array, would not be detected by NIPT or other diagnostic methods (aneuploidy screening, traditional karyotyping). However, all four conditions resulting from these chromosomal abnormalities are known to have variable penetrance.

**Conclusions:** In comparison with NIPT, SNP-array can potentially identify additional genetic abnormalities in fetuses with increased risk of common aneuploidies without ultrasound abnormalities. However, the variable penetrance of the additionally detected chromosomal abnormalities by SNP-array makes genetic counseling challenging.

phrosis. NIPT: high t18 probability.

Second case: 36-year-old primigravida, IVF-ICSI-pregnancy, mildly-increased nuchal-translucency. NIPT: normal male. Later sonogram demonstrated thickened nuchal-fold.

Third case: 28-year-old, normal first, second trimester screening, 15 week ultrasound: cardiac-echogenic-focus and choroid-plexus-cyst. NIPT: t21.

**Results:** First case: declined invasive testing, at delivery baby showed minor t18 signs, however lacked classic features. Neonate died of respiratory complications at 3 weeks. Cord-blood-karyotype: 46,XX,der(14)t(14;18) (p10;q10) (Partial t18).

Second case: Karyotype and CMA (amniocytes): atypical Klinefelter syndrome, with isochromosome-X.

Third case: amniocyte QF-PCR: normal, however, full-karyotyping: low level t21 mosaicism (50%).

**Conclusions:** Many factors limit NIPT accuracy. One main limitation is genetic discrepancy between fetal and placenta tissue. This is true in simple aneuploidies, moreover in incomplete/mosaic trisomies.

In the first case, although chromosome 18 only partially-duplicated, it was correctly identified by NIPT.

However, rare isochromosome Xq Klinefelter induced false-negative NIPT, consistent with lower performance of NIPT for detection of rare sex-aneuploidies. Xq-duplication probably causes even lower detection-rate than complete duplication.

For low-level mosaicism, NIPT detected a level of mosaicism of t21, below QF-PCR detection-threshold.

These unusual cases are examples that demonstrate NIPT strengths and weaknesses.

## P01.072

### False positive result of trisomy 13 by non-invasive prenatal testing (NIPT) due to confined placental mosaicism

C. C. Hung<sup>1</sup>, Y. N. Su<sup>1,2</sup>, H. W. Su<sup>3</sup>;

<sup>1</sup>Sofiva Genomics, Co., Ltd, Taipei, Taiwan, <sup>2</sup>Dianthus Maternal Fetal Medicine Clinic, Taipei, Taiwan, <sup>3</sup>Department of Obstetrics and Gynecology, Taichung Veterans General Hospital, Taichung, Taiwan.

**Introduction:** Recently, non-invasive prenatal testing (NIPT) has been proposed to be a powerful alternative to screening of aneuploidy with its high sensitivity and specificity. However, there is a small chance of false positive results due to placental mosaicism.

**Methods:** A 31-year old women went for NIPT at 19 weeks of gestation. The positive NIPT result was noted and Z-score value was 7.36 for chromosome 13. After informing the elevated risk for fetal trisomy 13, the pregnant woman accepted invasive diagnosis by amniocentesis to confirm the result. Amniotic fluid cells were used for karyotyping by GTG-banding and oligo array comparative genomic hybridization (aCGH).

**Results:** The karyotype was 46,XY and aCGH result showed arr[hg19] (1-22) x2, (X)x1, (Y)x1 on amniocentesis for the fetus. Following an abnormal NIPT result, both of the karyotype and aCGH genotype were inconsistent. Then the case underwent genotyping for the placenta and cord blood after childbirth. Interestingly, the genotype was arr[hg19] 13q12.11q34 (20,407,324-115,092,619)x3, (X)x1, (Y)x1 which is compatible with trisomy 13 for placenta, and had normal genotype for cord blood.

**Conclusion:** The American College of Obstetrics and Gynecology (ACOG) recommended that abnormal NIPT results should be confirmed by invasive procedures. This study show a false positive NIPT finding due to confined placental mosaicism because the fetal cell-free DNA present in maternal plasma is mainly derived from placenta.

## P01.073

### Fetal hydrops in combination with gonadoblastoid testicular dysplasia may represent a lethal type of Noonan syndrome

T. Reischer<sup>1</sup>, M. Schmid<sup>1</sup>, M. Nistal<sup>2</sup>, S. Balendran<sup>3</sup>, J. Vodopiutz<sup>4</sup>, E. Krampf-Bettelheim<sup>4,5</sup>, N. Popitsch<sup>6</sup>, S. Liebmann-Reindl<sup>7</sup>, B. Streubel<sup>8</sup>;

<sup>1</sup>Department of Obstetrics and feto-maternal Medicine, Medical University of Vienna, Vienna, Austria, <sup>2</sup>Department of Pathology, Hospital La Paz, Universidad Autónoma de Madrid, Madrid, Spain, <sup>3</sup>Department of Pathology, Medical University of Vienna, Vienna, Austria, <sup>4</sup>Department of Pediatrics, Medical University of Vienna, Vienna, Austria, <sup>5</sup>Fetomed - Center for Fetal Medicine, Doebling Private Hospital, Vienna, Austria, <sup>6</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, <sup>7</sup>Core facility genomics, Medical University of Vienna, Vienna, Austria.

**Objectives:** Fetal gonadoblastoid testicular dysplasia is a rare finding. The combination with typical features of Noonan syndrome has never been described so far. We performed genetic testing including whole exome sequencing in two cases with fetal hydrops, congenital heart disease and gonadoblastoid testicular dysplasia.

**Methods:** Exome sequencing was performed in the index case, where high quality DNA was isolated from fetal blood. In the second case and in five

## P01.071

### A free fetal DNA - Real Time PCR based - approach for prenatal diagnosis of beta globin gene mutations in embryos at risk for Thalassaemia

F. Strikou, L. Ikonomopoulos, A. Floratos, C. Chassanidis, E. Voskaridou, A. Balassopoulou, E. Boutou;

Laiko Hospital, Athens, Greece.

Thalassaemias, the most common genetic disorders worldwide, are inherited in a recessive autosomal manner. Therefore, prenatal diagnosis of embryos at risk presents the most common method of disease prevention and is predominantly based on genetic analysis of cells of fetal origin. This material is mainly obtained through invasive procedures with 1-2% probability of miscarriage. Therefore, non-invasive prenatal testing (NIPT) approaches, based on analyzing fetal DNA circulating in the maternal plasma, were developed.

Here, we present a novel approach for detecting beta globin gene mutations present in free fetal DNA (ffDNA). This method is based on High Resolution Melting (HRM) combined with quantitative analysis.

ffDNA was isolated from maternal serum (Qiamp method) and screened for the presence of  $\beta$  globin gene parental mutations based on an in-house HRM developed approach. In addition, previously described SNPs in the vicinity of  $\beta$  globin gene, for which parents presented distinct haplotypes, were also determined.

Preliminary results from 12 cases of male embryos carrying the paternal mutation succeeded in obtaining the same diagnosis with the preceded analysis of corresponding chorionic villus sampling. Interestingly, the presence of SRY gene was clearly detected in mixtures of 0,5% of male with female genomic DNA while ffDNA is estimated to be no less than 3% of the maternal free DNA.

The described approach may be directly applied for NIPT of cases where the embryo is male and has inherited the paternal  $\beta$  globin gene mutation. Further development of the method is expected to cover the rest of the cases.

## P01.074

### Non-invasive prenatal testing (NIPT) in unusual aneuploidy cases: partial trisomies and low-level mosaicism

R. Michaelson-Cohen;

Shaare Zedek Medical Center, Hebrew University, Jerusalem Israel, Jerusalem, Israel.

**Background:** NIPT for aneuploidies is extremely efficient, particularly for trisomy 21(t21) and t18, with sensitivity and specificity >99%. Clinical utilization of NIPT has grown rapidly.

**Patients and methods:** In our referral center, women undergo NIPT for several indications. We describe performance in 3 cases.

Three rare aneuploidies were diagnosed combining NIPT (USA-based-laboratories), karyotyping.

First case: 42-year-old woman, integrated-first-second-trimester screening: high t18 risk. Ultrasound: atrioventricular septal defect and hydrone-

further gonadoblastoma samples, conventional Sanger sequencing was performed on DNA isolated from formalin fixed, paraffin embedded tissue.

Results: Whole exome sequencing of the index case revealed a pathogenic mutation in the RIT1 gene (c.270G>A (p.Met90Ile)), leading to the diagnosis of Noonan syndrome type 8. In case 2, Sanger sequencing of RIT1 did not show any disease causing mutations. Sequencing of PTPN11 revealed a heterozygous frameshift mutation (c.1098delA, p.K366Nfs\*12). Successful sequencing of four gonadoblastoma samples revealed mutations in RIT1 in two cases.

Conclusions: Here we present a lethal form of Noonan syndrome in two fetuses with typical features combined with severe hydrops and gonadoblastoid testicular dysplasia. Gonadoblastoid testicular dysplasia may be an additional feature of Noonan syndrome, which has not been described so far, but analysis of more cases is needed. Moreover, we have demonstrated a potential role of RIT1 in the pathogenesis of gonadoblastoma.

#### P01.074

##### Azoospermia and Varicocele in Noonan Syndrome

F. Hazan<sup>1</sup>, E. Pariltay<sup>2</sup>, B. S. Ada<sup>3</sup>, E. Ataman<sup>2</sup>, A. Tukun<sup>3</sup>;

<sup>1</sup>Medical Genetics, Dr. Behcet Uz Children's Hospital, Izmir, Turkey, <sup>2</sup>Ege University Faculty of Medicine, Medical Genetics, Izmir, Turkey, <sup>3</sup>Duzen Laboratories Group, Division of Medical Genetics, Ankara, Turkey.

Noonan Syndrome (NS) is a syndrome having autosomal dominant inheritance, characterized by typical facial dysmorphic features, short stature and cardiac abnormalities. PTPN11, SOS1, RAF1, KRAS, NRAS, and BRAF are the genes responsible for this syndrome, in which genetically heterogeneity is considered. PTPN11 gene mutation is seen approximately in 50% of the patients. Although fertility seems normal in females, males have decreased fertility. The incidence of undescended testes (UT) ranges between 60-77% that is the most responsible cause of impaired male fertility. Besides the studies reporting the functional impairment of sertolian cells, according to the hormonal evaluation of NS during adulthood; there are studies remarking the functional impairment of leydig cells. The seminal analysis of male patients with NS was reported in 3 studies (8 patients). Three of these cases were normal, whereas half of them having azoospermia and one with severe oligospermia. Here we present a father having PTPN11 mutation, screened after having a child diagnosed as NS. Father had normally placed testicles, azoospermia and bilateral varicocele in his history. He had a child with intra-cytoplasmic sperm injection (ICSI) practice, after varicocele operation. As far as we know, this is the only NS case had the ICSI practice in the literature. In this page we report the clinical presentation of the father and his girl case with literature review.

#### P01.075

##### Findings of Oligo array CGH study of de novo small supernumerary marker chromosomes detected prenatally

H. Zolghadr, A. Rajaei, A. Moshtagh, N. Sadatian, G. Abbasi, m. bazregar, s. gholami, A. Kariminejad, R. Kariminejad;

Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran, Islamic Republic of.

One of the most problematic findings in prenatal chromosomal studies is the presence of non-hereditary small supernumerary marker chromosomes. It is common practice to identify the status of these chromosomes by performing routine C- and NOR banding. Recently we have encouraged all couples to have oligo array comparative genomic hybridization testing for all not bisatellited markers.

Overall we have done it for eleven samples in the past two years. All eleven had markers that were not present in chromosomal study of either parent, the presence of genomic material was hard to detect and would fall into the category of sSMCs. In all these cases the sSMC was present in more than 15% of the studied cells.

We performed oligo array cgh testing on DNA extracted from chorionic villi samples or in many cases cultured amniotic fluid cells. Oligo array was performed on Illumina Cytochip 4X44 or 8X60 ISCA platforms according to manufacturer's protocol.

The results show normal genomic content in all but two cases that we detected pericentromeric genomic duplication. One showed a 13 Mb gain and the other, a 2 Mb gain on chromosome 8. Considering the size of the imbalance in the first case, the parents opted for abortion. In the second case only four genes were present in the region and the pregnancy is ongoing.

Our findings suggest that despite the small size of the marker chromosomes and their cytogenetically similar appearance, genomic analysis is necessary to help predict their possible role and phenotypic contribution.

#### P01.076

##### An intronic variant in TP73 is not associated with ovarian reserve or response markers in females undergoing assisted reproduction

D. Tohlob<sup>1,2</sup>, P. Pemberton<sup>3</sup>, L. Mohiyiddeen<sup>4</sup>, W. G. Newman<sup>5</sup>;

<sup>1</sup>Mansoura faculty of medicine, Mansoura, Egypt, <sup>2</sup>Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom, <sup>3</sup>Department of Clinical Biochemistry, Manchester Royal Infirmary, Manchester, United Kingdom,

<sup>4</sup>Department of Reproductive Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.

The p53 family members are involved in many important biological processes, including cell regulation and apoptosis. TP73 has been associated with the size of the follicular pool, ovulation rate and maintenance of genomic stability. In a recent Brazilian study, homozygosity for an intronic variant in TP73 (c.283-5855G>A, rs4648551) was associated with diminished ovarian reserve during the assessment of females undergoing *in vitro* fertilization (IVF) [1].

The aim of our study was to investigate whether this intronic TP73 variant was associated with the ovarian reserve markers: follicle stimulating hormone (FSH), antral follicle count (AFC), and anti-Mullerian hormone (AMH), and early outcome measures: number of eggs retrieved, and gonadotropin dose required to controlled ovarian hyper-stimulation and late outcome measures, including live birth rate.

We genotyped the TP73 variant by Taqman allelic discrimination assay in 603 females, attending a reproductive medicine unit for their first cycle of controlled ovarian hyper-stimulation for IVF/ICSI. We did not detect any significant differences ( $p<0.05$ ) in FSH, AFC, AMH, the number of eggs retrieved, the gonadotropin dose used or the live birth rate in women with different TP73 variant genotypes.

Our results indicate that there is insufficient evidence to support genotyping this variant to individualize the treatment protocols of females undergoing IVF/ICSI.

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#### P01.077

##### Novel sequence of Pregnancy-Associated Glycoprotein-like family (PAG-L) identified in the human genome

M. Majewska<sup>1</sup>, A. Lipka<sup>2</sup>, G. Panasiewicz<sup>2</sup>, M. Gowkielewicz<sup>2</sup>, M. Jozwik<sup>3</sup>, A. Bossowska<sup>1</sup>, B. Szafranska<sup>2</sup>;

<sup>1</sup>Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn, Poland, <sup>2</sup>Department of Animal Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Olsztyn, Poland, <sup>3</sup>Department of Gynecology and Obstetrics, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn, Poland.

Introduction: The PAG-L genes encode chorionic proteins belonging to aspartic proteinases(AP), together with pepsin A,C,F, renin and cathepsin D. Conservative structure of the AP genes comprises promoter, 9 exons and 8 introns named A-H. The aim was to identify unknown nucleotide sequences of the PAG-L gene family in the human genome.

Material and Methods: Human gDNA templates (N=6) were isolated from leucocytes. The gDNA templates were used for PCR of the PAG-L sequence with primers that should amplify the region from 5'UTR to exon 2 (with exon 1 and intron A), according to AP gene structures (769-1556 bp). After electrophoresis and UV-visualization, dominant amplicons (approx. 700bp) were gel-out purified, precipitated and sequenced in both directions. All chromatograms were analyzed with Geneious software. Obtained gDNA sequences were compared to PAG-L cDNAs identified by NGS (Illumina).

Results: Sequencing of amplicons (197-680bp) permitted to identify novel sequence of the human PAG-L (named hPAG-L). This region of exon 1 revealed homology (88-99%) only to the pepsinogen family (especially PepA) in the primates. The identified sequence of the entire intron A (486bp) shared high homology (72-100%) to different gDNA clones of homo sapiens and PepA in some other mammals, including 76% homology (exon 1) and 51%

homology (intron A) to the porcine PAG2 gene.

Conclusions: This is the first report describing sequence identification of the hPAG-L/PepA gene fragment (exon 1 and intron A). Further complex studies are required to discover the entire structure of the hPAG-L gene, as potential useful novel marker for genotyping.

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**P01.078****EvE: Reducing the cost of array-based preimplantation genetic diagnosis for chromosome rearrangements**A. R. Jones<sup>1</sup>, A. Corrigan<sup>2</sup>, S. Bint<sup>2,3</sup>, A. Davies<sup>2,3</sup>, P. Renwick<sup>3,4</sup>, C. Mackie Ogilvie<sup>3,4</sup>, J. Ahn<sup>1</sup>,<sup>1</sup>Clinical Bioinformatics, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>2</sup>Genetics Laboratories, Viapath, London, United Kingdom, <sup>3</sup>Guy's and St Thomas' Centre for Preimplantation Genetic Diagnosis, London, United Kingdom, <sup>4</sup>Genetics Department, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom.

**Introduction:** The Guy's and St Thomas' Centre for Preimplantation Genetic Diagnosis (PGD) offers PGD for carriers of balanced chromosome rearrangements.

Currently, the service uses an array CGH platform to detect imbalances, hybridising DNA derived from embryo biopsies against Promega DNA as a reference sample, prepared in the same way as the biopsy. As only a few cells are biopsied, whole genome amplification is required to generate sufficient detectable signal; however, this introduces noise, which is compounded by differences between quality and nature of biopsy and reference material. We have developed the embryo versus embryo algorithm (EvE) which enables the use of an *in silico* reference, and removes the need to prepare a reference sample for each embryo, significantly reducing labour and consumable costs.

**Method:** Two embryos are hybridised to an array. EvE takes the fluorescence intensity data for each embryo and for a stored reference embryo and performs an *in silico* hybridisation, which can be analysed within standard array CGH analysis software.

**Results:** EvE was validated by retrospective analysis of 74 abnormal embryos. Subsequently, EvE was trialled prospectively for three months (46 embryos). Overall, EvE demonstrated sensitivity >99% (95% CI 97.6-100) and specificity >99% (95% CI 98.4-100).

**Conclusions:** We have validated and successfully completed a prospective trial using EvE for chromosome rearrangement PGD, which increases efficiency and reduces costs for this test. Furthermore, as an optimal reference can be used for each embryo, there is potential to improve quality and analytical sensitivity.

**P01.079****Preimplantation genetic diagnosis by aCGH in woman with familial complex translocation - 46,XX,t(3;6;7)**S. P. Hadjidekova<sup>1,2</sup>, G. S. Stamenov<sup>2</sup>, S. Y. Yaneva Staykova<sup>2</sup>, B. B. Rukova<sup>1,2</sup>, R. G.Staneva<sup>1,2</sup>, K. S. Nikolova<sup>2</sup>, D. I. Toncheva<sup>1</sup>,<sup>1</sup>Department of Medical Genetics, Medical University-Sofia, Sofia, Bulgaria, <sup>2</sup>"Nadezhda" Women's Health Hospital, Sofia, Bulgaria.

**Introduction:** Complex chromosome rearrangements (CCRs) are very rare in humans. In three-breaking-point translocations the hexavalent meiotic configuration allows four patterns of segregation: 3:3, 4:2, 5:1 and 6:0. 64 different gametic chromosomal combinations can arise of which only 2 are normal/balanced. Carriers have an increased risk of producing embryos with unbalanced karyotype. PGD enables the selection of balanced/normal embryos for transfer, enhancing the possibility for a healthy child.

**Materials and methods:** A couple suffered from two early miscarriages. The woman was found to carry CCR:

46,XX,t(3;6;7)(3pter→3q13.2::7p22→7pter;6pter→6q21::3q13.2→3qter;7qter→7p22::6q21→6qter). Her first cousin had the same translocation. The couple went through stimulated and Natural-Cycle-IVF. Embryo biopsy was carried out on Day 5, collecting 5-7 trophectoderm cells. The cells were amplified by whole-genome amplification through SurePlex. The amplification products were processed by 24sure+ microarrays (Illumina) and analyzed by BlueFuseMulti software.

**Results:** In total 36 ova were retrieved and two ICSI-PGD procedures were performed. From the first cycle 9 ova were aspirated and 4 embryos were biopsied, none was normal/balanced. The second ICSI-PGD procedure has started with 27 eggs, 11 embryos were biopsied. We found three normal/balanced embryos. Two were transferred on Day 6 after fertilization. The third normal/balanced embryo was frozen for future transfer. The women conceived with one embryo. She refused prenatal diagnosis but after birth the cytogenetic analysis confirmed normal male karyotype 46,XY.

**Conclusion:** The application of aCGH for PGD is an efficient test for CCR carriers. It reduces the risk of chromosomally imbalanced offsprings, miscarriages and enhances the baby take home rate.

**P01.080****An efficient protocol for the detection of junction fragments in PGD (preimplantation genetic diagnosis) for gross deletion mutations**

S. Peleg-Schalka, V. Gold, T. Frumkin, A. Carmon, S. Assaf, A. Amit, M. Malcov;

Racine IVF Unit, Lis Maternity Hospital, Sourasky Medical Center, Tel Aviv, Tel Aviv, Israel.

**Introduction:** In PGD for monogenic disorders the causal mutation is usually diagnosed by restriction enzymes or GeneScan analyses. However, in familial gross deletions,

primarily diagnosed by MLPA or SNP arrays, the exact breakpoints location is unknown and identifying the deleted region is challenging.

**Aim:** To present an effective and simple protocol to detect gross deletions in PGD by amplifying junction fragments.

**Methods:** Four couples carrier of gross deletions in chromosomes 11, 16, FANCA and MSH2 opted for PGD. Deletions ranged between 6-241kbp. In order to confine the junction fragments 6-25 primers, flanking the deleted regions, were purchased. The closest primers were designed 500bp from the positive probe/ SNP, followed by several primers in a similar distance. Following amplification of a junction fragment, amplicons' sizes were restricted by moving toward the breakpoints. **Results:** In 3 cases we managed to create a junction fragment and applied it to PGD. Amplification failure in one

case was due to extended duplication regions homologues to the deletion. Six PGD cycles were performed. One couple with deletion in chromosome 16 achieved pregnancy and another couple with simultaneous detection of 2 FANCA mutations and HLA matching has delivered one healthy child and BMT was performed successfully. **Discussion:** For maximal accuracy in PGD, direct detection of mutations along with haplotype analysis is required. Whenever informative polymorphic markers are absent in the deleted regions, we applied an effective protocol for junction fragments detection by scanning large DNA regions. This turns PGD for gross deletions a reliable and simple procedure.

**P01.081****Benefits of combined preimplantation genetic screening and endometrial receptivity assessment on IVF outcome for complicated reproductive history patients**L. Volozonoka<sup>1,2</sup>, L. Kornejeva<sup>2</sup>, N. Novikova<sup>3,2</sup>, V. Fodina<sup>2</sup>;<sup>1</sup>Riga Stradiņu University, Riga, Latvia, <sup>2</sup>"IVF Riga" Reproductive Genetics Clinic, Riga, Latvia, <sup>3</sup>Latvian University, Faculty of Medicine, Riga, Latvia.

**Introduction:** The mechanism of recurrent implantation failure (RIF) still remains a mystery in many cases of IVF treatment. Embryonic factor exclusion by preimplantation genetic screening (PGS) is relevant step in achieving considerably good results; however failure rates do not fade away. Testing of endometrial transcriptome (ERA-test) has been launched recently to catch skewed implantation window.

The aim of our study was to evaluate the endometrial receptivity as an implantation failure factor in infertile couples after negative post-PGS embryo transfer (ET).

**Materials and methods:** IVF was performed by ICSI technology. PGS was applied to 80 families. Trophectodermal cells were analyzed according to 24Sure protocol (Illumina). Endometrial biopsies for ERA-testing were performed according to manufacturer's (Igenomix) instructions.

**Results:** 19 couples (23%) had no euploid embryos after PGS (female age 41.06±3.42). 20 families are waiting for ET; 41 ET has been performed, 25 of them resulted in clinical pregnancies (61%, female age 33.77±5.14, BC amount 5.58±3.22, euploid embryo amount 2.33±1.55). In control group (679 couples, female age 38.25±5.4, no PGS, no ERA) clinical pregnancy rate was 37.86%. Half of the patients with negative ET post factum showed non-receptive endometrium.

**Conclusions:** Results suggest that PGS analysis can exclude one of the leading infertility causes - embryo chromosomal incompetence. Great amount of RIF patient has skewed implantation window, assessment of which is crucial in successful implantation establishment. RIF patient subgroup is heterogeneous and options such as PGS should be applied carefully based on embryo amount and quality, managing infertile couples in individual patient-tailored manner.

## P01.082

### Uniparental disomy of chromosome 12 which leads to a phenylketonuria case

*S. Azadmehr<sup>1</sup>, T. Shirzad<sup>1</sup>, E. Davoudi-Dehaghani<sup>1</sup>, S. Dabbagh-Bagheri<sup>1</sup>, Z. Sharifi<sup>1</sup>, A. Bandehi Sarhadi<sup>1</sup>, L. Rejali<sup>1</sup>, F. Mollazade<sup>1</sup>, F. Golnabi<sup>1</sup>, H. Bagherian<sup>1</sup>, M. Fallah<sup>1,2</sup>, S. Zeinali<sup>1,3</sup>*

<sup>1</sup>Dr. Zeinali's Medical Genetics Lab, Kawsar Human Genetics Research Center (KHGRC), Tehran, Iran, Islamic Republic of, <sup>2</sup>Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>3</sup>Department of Molecular Medicine, Biotech Research Center, Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of.

**Introduction:** Phenylketonuria (PKU) is one of the most prevalent amino acid metabolism disorders caused by deficiencies in the PAH gene located on chromosome 12(12q23.2). Affected persons are born from carrier parents with autosomal recessive pattern of inheritance.

**Material and method:** A 9 -year -old boy who was identified to be affected through newborns screening for PKU referred to our laboratory. Sanger sequencing of PAH gene was performed by extracted DNA from peripheral blood sample. Linked STRs and VNTR analysis were done in parallel. MLPA (Multiplex ligation-dependent probe amplification) technique was utilized to detect probable deletion in PAH gene. Performance of DNA fingerprinting by evaluation of 16 different DNA markers for the child and his mother.

**Result:** PAH: c.782G>A mutation was detected in patient. His father was heterozygote for the same mutation while surprisingly his mother was normal. DNA fingerprinting confirmed maternity with over 99.9% certainty. MLPA results did not show any deletion for the affected child and also his mother. No common haplotype in STR analysis and same number of repeats in VNTR was found for the affected child and his mother. The probability of uniparental disomy of chromosome 12 was verified observing homozygous haplotype in STR analysis linked to two other genes on chromosome 12, VWF gene 12p13.3 and MMAB gene 12q24.

**Conclusion:**

Paternal uniparental disomy of chromosome 12 is a rare chromosomal anomaly. This study highlighted the importance of STRs and VNTRs analysis along with mutation detection to specify necessity of prenatal diagnosis.

## P01.083

### Study of the genetic etiology of primary ovarian insufficiency (POI): FMR1 and FMR2 genes

*M. Barasoain<sup>1</sup>, G. Barrenetxea<sup>2</sup>, I. Huerta<sup>1,3</sup>, M. Télez<sup>1,3</sup>, E. Ortiz-Lastra<sup>2</sup>, J. González<sup>2</sup>, B. Criado<sup>4</sup>, M. Arrieta<sup>1</sup>*

<sup>1</sup>Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country, Bilbao, Spain, <sup>2</sup>Department of Medical-Surgical Specialities, Faculty of Medicine, University of the Basque Country, Bilbao, Spain, <sup>3</sup>Virgen de Begoña Clinical Analysis Laboratory (Medikosta), Erandio, Spain, <sup>4</sup>Cooperativa de Ensino Superior Politecnico e Universitario (CESPU), Porto, Portugal.

Primary ovarian insufficiency (POI) is an ovarian dysfunction defined as irregular menses and elevated gonadotrophin levels before or at the age of 40 years. The FMR1 intermediate (35-54 CGGs) and premutation (55-200 CGGs) alleles have been related with the development of this condition. The FMR2 alleles with 11 or less CCG repeats are also a possible genetic cause of POI. A group of 68 women with ovarian dysfunction of unknown etiology, divided into three groups concerning their ovarian condition, and 47 control women from the Basque Country were analyzed. The FMR1 gene, including the length of the repeat and the AGG interspersion pattern, and the number of CCG repeats of the FMR2 gene were evaluated. Considering the FMR1 gene, the frequency of alleles with 35-200 CGG repeats was statistically higher in the patient group and in the three subgroups. Moreover, these alleles appeared to have two interruptions, the first AGG located in the 10th position and more than 15 CGG at the 3' end with the predominant structure 9+9+n. The patient group presented, in general, more unstable alleles and therefore the FMR1 gene appeared to play a role in the etiology of POI in the patient group. Regarding the FMR2 gene, we have not found a clear association with the development of an ovarian dysfunction.

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## P01.084

### Isomerism, intellectual disability and clinical features associated with mutations in the Polyglutamine-binding protein 1 (PQBP1) gene

*L. Martínez Ribot<sup>1</sup>, P. Fernández Álvarez<sup>1</sup>, M. Sánchez Duran<sup>2</sup>, S. Arévalo Martínez<sup>3</sup>, T. Vendrell Bayona<sup>1</sup>, A. Cueto González<sup>1</sup>, M. Borregan Prats<sup>1</sup>, F. Lopez Grondona<sup>1</sup>, E. Tizzano Ferrari<sup>1</sup>*

<sup>1</sup>Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron Barcelona, Spain, Barcelona, Spain, <sup>2</sup>Prenatal Diagnosis Unit, Hospital Vall d'Hebron Barcelona, Spain, Barcelona, Spain, <sup>3</sup>Fetal Medicine Unit, Hospital Vall d'Hebron Barcelona, Spain, Barcelona, Spain.

**Introduction:** A 31-year-old woman was referred to our center at 20 weeks of gestation because of a male fetus presented congenital heart defect, left isomerism and ventriculomegaly. No consanguinity was reported but as family history, her brother and a maternal uncle had mental retardation of unknown cause and similar physical characteristics.

**Methodology:** Genetic prenatal studies performed in the fetus (karyotype and CGH-array) were normal. The differential diagnosis of the fetus was interpreted not only based in the autopsy findings but also mental retardation present in the two males. PQBP1, an X-linked gene, was considered to be a potential candidate cause. Sanger sequencing of the coding region of PQBP1 (six exons, GenBank accession no. NM\_005710.2) as well as the flanking intronic sequences were performed from fetal genomic DNA and after in DNA of the consultant, her brother, mother, maternal uncle and aunt.

**Results:** A 5'UTR mutation (c.-20A>C; NG\_015967.1:g.5579A>C) was identified in the fetus, the patient, her mother and both males affected with mental retardation.

The mutation identified was not described before in the scientific literature. It is considered as possible pathogenic because its location in the promoter region of the gene could affect the transcription/ traduction of the protein. Apart from cognitive impairment, physical similarities were found in both males. All these features are described in patients with other PQBP1 mutations.

**Conclusions:** PQBP1 should be considered as a candidate to study when an X-linked unknown cause of mental retardation is associated with clinical features such as isomerism, short stature and microcephaly.

## P01.086

### The clinical utility of preimplantation genetic diagnosis with human leukocyte antigen matching (PGD-HLA): an ESHRE PGD Consortium multicentre retrospective cohort study

*J. Traeger-Synodinos<sup>1</sup>, G. Kakourou<sup>1</sup>, S. Kahraman<sup>2</sup>, G. C. Ekmekci<sup>3</sup>, H. A. Tac<sup>2</sup>, G. Kourlaba<sup>3</sup>, E. Kourkouni<sup>3</sup>, A. Cervero San<sup>4</sup>, J. M. Rodriguez<sup>4</sup>, A. Hellani<sup>5</sup>, H. Malmgren<sup>6</sup>, C. Giménez<sup>7</sup>, V. Gold<sup>8</sup>, F. Carvalho<sup>9,10</sup>, C. Billi<sup>11</sup>, J. F. C. Chow<sup>12</sup>, X. Vendrell<sup>13</sup>, G. Kokkali<sup>14</sup>, J. Liss<sup>15</sup>, J. Steffann<sup>16,17</sup>*

<sup>1</sup>Medical Genetics, National and Kapodistrian University of Athens, Athens, Greece,

<sup>2</sup>Istanbul Memorial Hospital, IVF and Reproductive Genetic Unit, Istanbul, Turkey,

<sup>3</sup>Collaborative Center for Clinical Epidemiology and Outcomes Research (CERO), 1st and 2nd Departments of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece, <sup>4</sup>IGenomix, Valencia, Spain, <sup>5</sup>Vialet, Dubai, United Arab Emirates, <sup>6</sup>Stockholm PGD Centre, Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden, <sup>7</sup>Reprogenetics, Barcelona, Spain, <sup>8</sup>PGD Lab, Racine IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, <sup>9</sup>Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal, <sup>10</sup>IS3 - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal, <sup>11</sup>AlfaLab Molecular Biology and Cytogenetics Center, Athens, Greece, <sup>12</sup>Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong, <sup>13</sup>Sistemas Genómicos Ltd, Reproductive Genetics Unit, Valencia, Spain, <sup>14</sup>Reproductive Medicine Unit, Genesis Athens Clinic, Athens, Greece, <sup>15</sup>INVICTA Fertility Centre, Warsaw, Poland, <sup>16</sup>Paris-Descartes University, Imagine Institute, Paris, France, <sup>17</sup>INSERM U781, Necker-Enfants Malades Hospital, Paris, France.

Since the first cases of PGD-HLA in 2001, >1000 cases have been performed, making it a well-established procedure. However, limited publications preclude evaluation of parameters (assisted-reproduction, embryology, genetic probability) influencing positive outcomes: donor-live-born babies and haematopoietic stem-cell transplantation (HSCT). This retrospective multicentre cohort study, co-ordinated by the ESHRE PGD-Consortium, aims to address this. In 2014, 30 PGD centres with published/known PGD-HLA activity were invited to participate. Fourteen centres submitted data between February-September 2015, using a custom-designed secure database (Redcap). Data parameters covered assisted-reproduction procedures, embryology, genetic diagnosis, donor-babies born and HSCT for 704 PGD-HLA cycles (364 couples), analysed using STATA SEv.11. HLA-matching with concurrent exclusion of monogenic disease accounted for 81%. Mean maternal age was 33years, 7.5% couples were infertile, with 1.93 PGD- HLA cycles/couple. 9751 oocytes were retrieved (13.89/cycle), 5552 embryos (7.88/cycle) were analyzed (85% on day-3), using PCR-based protocols (97% cycles). Of 4392 embryos diagnosed (79% of analyzed), 644 were genetically suitable (16.2% of those analysed for HLA alone; 10% of those ana-

lysed for HLA with monogenic disease exclusion). 56.6% of couples had an embryo-transfer (598 embryos in 382 cycles), producing 163 HCG-positive pregnancies (pregnancy-rate/embryo-transfer: 42.67%, pregnancy-rate/initiated cycle: 24.3%). To September 2015, 127 babies were born and 30 pregnancies ongoing. HSCT was performed in 55 cases (7.8% cycles initiated), 76.1% without complications. This first multicentre study evaluates clinical utility of PGD-HLA. Genetic probability remains a major limitation to overall success, but further data analysis is expected to highlight other factors. Acknowledgement: ESHRE funded database customization and data analysis.

#### P01.087

#### Identification of mutated genes in consanguineous families with premature ovarian failure

*S. Caburet<sup>1,2</sup>, F. Fauchereau<sup>1,2</sup>, A. Todeschini<sup>1,2</sup>, B. Legois<sup>1,2</sup>, M. Fellous<sup>3,4</sup>, R. A. Veitia<sup>1,2</sup>;*

*<sup>1</sup>Institut Jacques Monod, Paris, France, <sup>2</sup>Université Paris Diderot-Paris VII, Paris, France,*

*<sup>3</sup>Institut Cochin, Paris, France, <sup>4</sup>Université Paris Descartes-Paris V, Paris, France.*

Premature ovarian failure is the endpoint of premature ovarian insufficiency (POI) and represents the complete loss of ovarian function. Although POI is a multifactorial condition, the existence of familial cases shows that it can be inherited as the result of a genetic defect. The genetic analysis of families, particularly very informative consanguineous families, is therefore invaluable to identify genes involved in the pathology.

We analysed two consanguineous families from Middle-East, respectively with 4 and 2 affected sisters. Combining linkage analysis, homozygosity mapping and exome sequencing, we identified in the first family a 1-bp deletion in the STAG3 gene, leading to a truncated coding sequence. STAG3 encodes a meiotic component of the synaptonemal complex, a ring that entraps sister chromatids and is required for proper pairing of homologous chromosomes during meiosis. STAG3 loss of function as the cause of POI was confirmed by the Stag3-deficient mouse model, in which both sexes were infertile due to loss of germ cells and degenerative gonads. In the second family, this approach highlighted a premature stop codon in MCM9, encoding the minichromosome maintenance complex component 9, recently implicated in POI associated with short stature. Furthermore, Mcm9-deficient mice were sterile with empty ovaries. Therefore, our results provided the first confirmation of the implication of MCM9 mutations in POI, expanding the phenotypic spectrum to females with normal height.

The genetic analysis of consanguineous families combining linkage analysis and exome sequencing is a powerful way to unravel genetic etiologies, especially in highly heterogeneous conditions such as POI.

#### P01.088

#### Current dilemmas in prenatal diagnosis revealed through new genomics applications and a single center's 30 years' experience and 90,000 cases

*C. Konialis, C. Pangalos;*

*InterGenetics-Diagnostic Genetics Center, Athens, Greece.*

Introduction: Recent developments in genomics and the wealth of data accumulated from the application of various diagnostic molecular approaches in prenatal diagnosis, combined with the advent of novel non-invasive prenatal screening tests (NIPT), have introduced new challenges in this highly sensitive field. We provide a perspective of prenatal diagnosis derived from a single center's evolving experience from more than 90,000 consecutive prenatal cases and we highlight important issues and current dilemmas.

Materials and Methods: Prenatal cases were referred for various indications and more than 90,000 clinical investigations were performed by a combination of standard karyotype, multiplex ligation-dependent probe amplification (MLPA), standalone array comparative genomic hybridization (aCGH) and exome sequencing.

Results: Classic karyotype revealed 1.7% and 7.9% of pathological cases in amniotic fluid and CVS samples, respectively, common aneuploidies accounting for 59.6% and 64.3% of the total abnormal. Molecular approaches increased the diagnostic yield by 0.6% for MLPA and 1.6% for aCGH, uncovering pathogenic chromosomal abnormalities undetectable by karyotype analysis. Finally, exome sequencing provided a further beneficial increase in our diagnostic capabilities.

Conclusions: Current molecular diagnostic capabilities and the recent introduction of NIPT point to one current major dilemma in prenatal diagnosis, with serious implications in genetic counseling of parents, relating on the one hand to reaping the benefits from the high detection rate afforded through new powerful diagnostic techniques, but accepting an invasive risk, and, on the other hand, offering a lower detection rate practically for Down syndrome only, with minimal invasive risk.

#### P01.089

#### Walker-Warburg syndrome as a cause of prenatally detected hydrocephalus in two Roma families

*J. Lastůvková<sup>1</sup>, A. Klímová<sup>1</sup>, M. Tóthová<sup>2</sup>, T. Nix<sup>2</sup>, M. Fiser<sup>2</sup>, V. Čejnová<sup>1</sup>, V. Harmaš<sup>1</sup>;*

*<sup>1</sup>Masaryk Hospital, Ústí nad Labem, Czech Republic, <sup>2</sup>GENvia, s.r.o., Praha, Czech Republic.*

Walker-Warburg syndrome is a rare autosomal recessive inherited condition characterised by brain and eye abnormalities and severe congenital muscular dystrophy. Most of affected individuals die before the third year of age. Disorder is caused by mutations in several different genes, one of them is ISPD gene.

Our patients are two unrelated young women of Roma origin. Both of them were referred to genetic examination because of ultrasound detection of hydrocephalus of foetus in the second trimester of gravidity. Diagnosis was also confirmed by magnetic resonance imaging. Both women decided to terminate their pregnancy. Karyotype of both foetuses was normal, but Array CGH identified homozygous a 290kb deletion of ISPD gene at chromosome 7, which is one of causes of Walker-Warburg syndrome. Mothers and their partners are heterozygous carriers of this deletion. Genetic counselling was provided in affected families. They were informed about possibility of assisted reproduction and PGD and about possibility of early prenatal detection of Walker-Warburg syndrome from CVS. Unfortunately, in the subsequent gravidity of both our patients the foetal hydrocephalus was again confirmed in the second trimester.

The overall incidence of Walker-Warburg syndrome is unknown, but for example in the Ashkenazi Jewish population it is 1 in 60500 with frequency of heterozygous carriers 1 in 149. There is no study about frequency of Walker-Warburg syndrome in Roma population.

Walker-Warburg syndrome should be considered in a differential diagnosis of prenatal detected hydrocephaly and it would be useful to assess frequency of ISPD gene deletion in Roma population.

#### P01.091

#### A QF-PCR/aCGH strategy for the diagnosis of chromosome imbalance in products of conception (POC) and fetal tissues

*C. Donaghue<sup>1</sup>, N. Davies<sup>1</sup>, J. Ahn<sup>2</sup>, H. Thomas<sup>1</sup>, C. Mackie Ogilvie<sup>2</sup>, K. Mann<sup>1</sup>;*

*<sup>1</sup>Genetics, VIAPATH, London, United Kingdom, <sup>2</sup>Genetics, Guys and St Thomas Hospital, London, United Kingdom.*

In the UK, karyotype analysis of couples with recurrent miscarriage has been replaced by genetic investigation of POCs and fetal tissues. As a consequence, sample numbers for these tissues have increased by 69% in our laboratory since 2012. We have replaced karyotype/MLPA testing of miscarriage products with QF-PCR for chromosomes 13, 14, 15, 16, 18, 21, 22, X and Y, followed in the case of normal results by aCGH. All array imbalances >1Mb and any fully penetrant clinically significant array imbalances <1Mb are reported. In an 18 month period, 1391 samples were tested using this approach. 25.9% of samples were found to be abnormal on QF-PCR and aCGH identified a further 9.4% of samples with clinically significant imbalance, giving an overall abnormality rate of 35.3%. The initial QF-PCR test is rapid, cost-effective and clinically indicated, detecting triploidy (4%) and maternal cell contamination in addition to the common aneuploidies. The aCGH analysis approach minimises the number of unknown and reduced penetrance CNVs that are reported; 0.7% of samples were reported to have an imbalance of uncertain clinical significance. Overall, our QF-PCR/aCGH strategy has a lower failure rate and higher detection rate than karyotype or MLPA strategies. Detection of chromosome imbalance provides reasons for pregnancy loss and fetal abnormalities, minimising further investigations and informing risk of recurrence of both miscarriage and fetal abnormality.

#### P01.092

#### Recurrent miscarriage and implantation failure-Could the etiology be maternal intolerance itself?

*P. Ata<sup>1</sup>, S. Soysal<sup>2</sup>, A. Turkyilmaz<sup>1</sup>, K. Delil<sup>1</sup>, G. Ilhan<sup>3</sup>, B. Geckinli<sup>1</sup>, M. Vural<sup>2</sup>;*

*<sup>1</sup>Dept of Medical Genetics, Marmara University Faculty of Medicine, Istanbul, Turkey,*

*<sup>2</sup>Dept of Obstetrics and Gynecology, Marmara University Faculty of Medicine, Istanbul, Turkey, <sup>3</sup>Dept of Obstetrics and Gynecology, Marmara University Faculty of Medicinie, Istanbul, Turkey.*

Recurrent miscarriage and recurrent implantation failure (RIF) is one of the most controversial issues in assisted reproduction. Herein our aim is to search the factors affecting maternal tolerance to pregnancy.

We investigated 34 infertile couples with recurrent miscarriage, admitted to assisted reproduction between July 2014 to January 2016. Male factor is excluded. Karyotyping and thrombophilic mutations (Prothrombin, Factor V Leiden, MTHFR 677, 1298 mutations) were analyzed. HLA and KIR typing

of the couples and Panel Reactive Antibody (PRA) level of the females were performed. For HLA and KIR typing Sequence Specific Primer (SSP) technique is used. Natural Killer Cell quantitation and PRA levels are determined by Flowcytometric analysis.

Of the 34 couples we had 10 couples who had three and more miscarriages and implantation failure and the remaining 24 couples had one or two miscarriages after assisted reproduction. There were more patients with MTHFR mutations at the second group than the first ( $p < 0.05$ ). There were no patients with Factor V Leiden and Prothrombin mutations. Five couples had chromosomal rearrangements of three inversion chromosome 9 and two translocations. Patients at the second group having more miscarriages had maternal KIR typing as AA haplotype and paternal HLA-C2 typing as predisposing factor for the "fetal rejection" compared to the first group patients (8/10 versus 5/24 respectively,  $p < 0.05$ ).

Herein our results support that there is a worse effect of KIR and HLA-C mismatch on fertility and those patients with MTHFR mutations had more miscarriages compared to wild type MTHFR carriers.

#### P01.093

#### Change to guidelines for investigations for recurrent miscarriage - effect on solid tissue referrals

N. Davies, C. Donaghue, R. Pain, L. Allan, A. Bergbaum, K. Mann, K. Pawliczak; Viapath, Guy's Hospital, London, United Kingdom.

In April 2011 the Royal College of Obstetricians and Gynaecologists (RCOG) published new recommendations for investigation and treatment of couples with recurrent miscarriages; cytogenetic analysis should now be performed on products of conception rather than the couple's blood samples. Inheritance studies are only recommended if an unbalanced structural chromosomal abnormality is detected in the products of conception.

These recommendations were implemented in the SE Thames region in April 2012.

We experienced an increase in the total number of products of conception samples from 2011/12 to 2012/13 (42.3%) and a further increase from 2012/13 to 2013/14 (18.4%), with concomitant increase of products with sample quality insufficient for analysis.

Gestation was given for 1126 (61%) samples; the first trimester samples increased from 109 in 2011/12 (39.4%) to 181 in 2012/13 (51%) to 239 in 2013/14 (52.1%). Of the samples received, with a known gestation, following the release of the new guidelines, 207 (11.3%) samples were classed as unsuitable, of which 71 were from the first trimester. Overall, the number of tests which did not yield a result rose from 9.6% in 2012/13 to 12.3% in year 2013/14.

Poor morphology, or lack of fetal or placental material in these early gestation samples are the likely causes of the increase in failure to obtain a diagnosis. Nevertheless, cause of miscarriage was identified in 35.3% of cases, in the period following the new recommendations.

#### P01.094

#### Relevance of SNP on p53, IL-11, IL-10, VEGF and APOE in patients with repeated implantation failure (RIF) and pregnancy loss (RPL)

B. Lledo<sup>1</sup>, J. Ortiz<sup>1</sup>, R. Morales<sup>1</sup>, A. Turienzo<sup>1</sup>, J. Llacer<sup>2</sup>, R. Bernabeu<sup>1</sup>;

<sup>1</sup>Instituto Bernabeu Biotech, Alicante, Spain, <sup>2</sup>Instituto Bernabeu, Alicante, Spain.

**INTRODUCTION:** In a population of RIF and RPL patients a high incidence on different gene polymorphisms was observed. The aim of this work was investigate whether single nucleotide polymorphism on p53, IL-11, IL-10, VEGF and APOE have a higher prevalence among women with a history of recurrent implantation failure (RIF) and pregnancy loss (RPL).

**MATERIAL AND METHODS:** SNPs genotyping (rs1042522-p53-R72P; rs1800896-IL-111082AG; rs1570360-VEGF-1154AG; rs11668344-IL-10; rs429358-APOE-R112C; rs7412-APOE-R158C) using TaqMan-assays has been studied in 255 women. The control group included 89 oocyte donors. In the study group included 166 women: 77 with RIF and 89 with RPL. RIF was defined as a total of four transferred cleaved good quality embryos with negative hCG. RPL was defined as two or more miscarriages.

**RESULTS:** The frequency of P72/P72 genotypes on p53 gene among women experiencing RIF was 9.5% compared with 13.5% for those with a history of RPL and 6% in controls ( $p < 0.05$ ). The frequency of E4 isoform on APOE gene among RIF was 18.6% compared with 28.9% for those with RPL and 13.5% in controls ( $p < 0.05$ ). A tendency between the frequency of genotypes distribution with respect to VEGF and IL-11 was shown with no statistical significance difference. As for IL-10 no differences were reported.

**CONCLUSION:** This investigation reveals that in RIF and RPL patients P72 on p53 gene and E4 isoform on APOE are more prevalent than fertile population. This information together with some additional markers will allow developments of diagnostic tests for detect risk for RIF and RPL before infertility treatment is initiated.

#### P01.096

#### Fetal imaging in the diagnosis of skeletal dysplasias and craniosynostosis - a case series

V. Ward<sup>1</sup>, M. Irving<sup>1</sup>, A. Beleza<sup>1</sup>, S. Sankaran<sup>2</sup>, P. Kyle<sup>2</sup>, G. J. Lee<sup>3</sup>, C. Walsh<sup>4</sup>, S. Allouni<sup>4</sup>, C. Ogilvie<sup>5</sup>, K. Mann<sup>5</sup>, D. Shears<sup>6</sup>, J. Carmichael<sup>7</sup>, M. Holder-Espinasse<sup>1</sup>;

<sup>1</sup>Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>2</sup>Maternal-Fetal Medicine, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>3</sup>Neonatology, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>4</sup>Molecular Genetics, Viapath, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>5</sup>Genetics laboratories, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>6</sup>Clinical Genetics, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, <sup>7</sup>Paediatric Radiology, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom.

There are more than 450 skeletal dysplasias ranging in severity and degree of associated complications, and although individually rare, collectively they have a birth incidence of around 1 in 5000. Prenatal diagnosis is often prompted by the finding of short long bones on ultrasound scanning or in cases with a family history of skeletal dysplasias. Specific diagnosis can be difficult and relies on clinical, radiological and molecular criteria.

Craniosynostosis, the premature fusion of cranial sutures, has a prevalence of 1 in 2000 and around 15% of these are syndromic and associated with other anomalies. Prenatal ultrasound diagnosis of craniosynostosis can be difficult and so a definite diagnosis relies on DNA testing.

The use of fetal MRI and CT scan imaging has advantages over ultrasound imaging and can provide excellent tissue resolution and a greater field of view. Detailed imaging allows for a clear differential diagnosis to be established as early as possible, enabling further investigations such as molecular diagnostic testing to be considered. This can allow for the distinction between lethal and non-lethal dysplasias, and permit further discussions with parents around prognosis and management.

We report 4 prenatal cases of Saethre-Chotzen and Carpenter syndromes, Hypochondrogenesis, and Metatropic dysplasia with their clinical, radiological and molecular features. We show the first UK case where fetal CT scan was performed as well as MRI images. The findings demonstrate the importance of fetal imaging in helping to reach a prenatal diagnosis and the impact it has on subsequent clinical management.

#### P01.097

#### QF-PCR and SNP array offer a superior method for detection of copy number variant and copy number neutral abnormalities in a consanguineous family

G. A. M. Nimmo<sup>1</sup>, N. Martin<sup>2</sup>, A. Staines<sup>2</sup>, N. Watkins<sup>3</sup>, A. Noor<sup>3</sup>, D. Chitayat<sup>1,2</sup>, E. Kolomietz<sup>2</sup>;

<sup>1</sup>Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, The University of Toronto, Toronto, ON, Canada, <sup>2</sup>The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada, <sup>3</sup>The Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada.

**Background:** Chromosome microarray analysis can identify submicroscopic genomic imbalances that may not be detectable by karyotyping. Because of this increased yield, microarray analysis is emerging as a first-line test for prenatal diagnosis in pregnancies with fetal anomalies or with invasive testing for another indications. Furthermore, single nucleotide polymorphism (SNP)-arrays offer the ability to detect copy neutral chromosomal aberrations such as uniparental disomy and absence of heterozygosity. Our centre offers combined rapid aneuploidy screening, by quantitative fluorescence-PCR (QF-PCR), with SNP-array as a first line test in women undergoing invasive prenatal testing.**Report:** We describe a consanguineous couple, known to be carriers for a recessive disease by family history, presenting with multiple fetal abnormalities at 12 weeks gestation. The fetal abnormalities were not in keeping with disease known to segregate within this family. Combined aneuploidy screening performed on chorionic villus sampling was suggestive of monosomy 21. SNP-array subsequently demonstrated normal chromosome copy-number and near complete absence of heterozygosity (AOH) of chromosome 21. Further testing revealed homozygous mutations in the *MKS1* gene known to cause Meckel-Gruber Syndrome.**Discussion:** The initial QF-PCR result of monosomy 21 would have predicted a low recurrence risk for this family. SNP-array demonstrated a significant proportion of the genome with AOH. This information, along with the findings from additional molecular testing identified that this couple was a carrier for a second recessive disease. The combination of both tests allowed for more accurate counselling in this and future pregnancies.

**P01.098****Prenatal diagnosis of a de novo, interstitial duplications of 10q24.32, associated with split hand and foot malformation by array comparative genomic hybridization***M. Smyk<sup>1</sup>, P. Węgrzyn<sup>2</sup>, K. Sobcka<sup>1</sup>, M. Kędzior<sup>1</sup>, E. Obersztyn<sup>1</sup>, B. A. Nowakowska<sup>1</sup>;**<sup>1</sup>Dep. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland, <sup>2</sup>Dep. of Obstetrics and Gynecology of the Medical University of Warsaw, Warsaw, Poland.*

**Introduction:** Split hand and foot malformation (SHFM) is a condition characterized by hypoplasia or aplasia of central digital rays, clefts of hands and feet and variable fusion of remaining digits and occurs in 1:20000 newborns. In 2004 Roscioli et al. characterized a large autosomal dominant pedigree segregating split-hand/foot linked to chromosome 10q24. Since then postnatal cases with duplication of this region and SHFM phenotype have been described. Herein we present for the first time two prenatal cases of a de novo interstitial duplication of 10q24.32 and SHFM.

**Materials and Methods:** In both cases amniocentesis was performed at 16 weeks gestation, due to the presence of fetal SHFM, detected by ultrasound scan. Chromosome analyses were performed on cultured amniotic cells at approximately 350 band resolution. Array CGH was performed using commercially available array (CytoSure, OGT, UK). Confirmatory FISH analyses were performed in cultured amniotic cells and peripheral blood lymphocytes from parents, using BAC clone specific for the 10q24.32 region.

**Results:** G-band chromosome analyses revealed normal karyotypes, whereas the array CGH detected duplication at chromosome 10q24. In the common duplicated region 3 genes are mapped: BTRC, POLL and FBXW4, the results were confirmed by FISH.

**Conclusions:** The detailed second trimester ultrasound scan can detect major fetal malformations and is offered for routine prenatal care. Array-CGH allowed a precise chromosomal diagnosis, confirming this analysis as a first tier approach to clarify molecular bases of fetal malformations.

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**P01.099****Preimplantation diagnostics after genomic analysis in spontaneous abortions***I. Dimova<sup>1</sup>, T. Milachich<sup>2</sup>, M. Savova<sup>2</sup>, T. Timeva<sup>2</sup>, M. Yunakova<sup>2</sup>, A. Shterev<sup>2</sup>;**<sup>1</sup>Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>SAGBAL Hospital „Dr.Shterev“, Sofia, Bulgaria.*

Spontaneous abortions, defined as embryonic or fetal loss before 20 weeks of gestation, occur at a frequency of 10-15% of confirmed pregnancies. Establishing their etiology is essential for proper clinical decision in each case. About 10% of women with one miscarriage are at risk of recurrent spontaneous abortions, which is a great physical and emotional burden. The most common causes of this condition are fetal genetic abnormalities. In the present study we performed whole-genome analysis by array CGH in 10 abortive tissues. We detected the following genomic anomalies: 2 cases with 47,XY,+22 [arr(22)x3]; 1 case with 47,XX,+20 [arr(20)x3], 47,XY,+16 [arr(16)x3] and 46,XY,arr(7p)x3. Since the last case represents a structural aberration we assumed a carriership of balanced chromosomal aberration and performed cytogenetic analysis of the parents. The karyotype of the mother showed balanced translocation with breakpoints 7q11 and 10p13 - 46,XX,t(7;10)(q11;p13), explaining the observed fetal anomaly. Based on this result we performed preimplantation diagnostics in the family using array CGH and only balanced embryo was transferred. The patient has successful ongoing pregnancy.

**Conclusion:** We detected chromosomal aberrations in 50% of the analyzed cases. Among them were present both frequent (such as trisomy 16 and 22) and less common (trisomy 20) anomalies. The structural anomaly revealed constitutional aberration which needs PGD for patient's safety and successful pregnancy. We conclude that genomic analysis is an effective tool in the complex diagnostic approach in spontaneous abortions and could be first line analysis with great impact on decision making.

**P01.100****Recurrent de novo translocation trisomy 21 in the same family***I. Soldatova, J. Horáček, E. Zemáňová, M. Klášová, D. Stejskal, M. Koudová;**GENNET, Prague, Czech Republic.*

Rapid detection of aneuploidies of chromosomes 13,18, 21, X and Y (RAD) using Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) has been routinely performed in our laboratory since 2002.

Here we present a case of recurrent translocation trisomy 21 (Down syndrome) in one family resulting from two different maternal meiotic division errors. In both cases origin and mechanism of non-disjunction was elucidated by the QF-PCR.

The first pregnancy of unrelated healthy parents (25 and 24 years) was referred to invasive prenatal diagnosis due to significant aneuploidy risk calculated from multi-marker screening results in 2010. Diagnostic procedure was declined by the family and the newborn karyotype was 46,XX,+21,der(21;21)(q10;q10). Both parental karyotypes were normal (46,XX and 46,XY). QF-PCR using peripheral blood of the newborn proved maternal origin of trisomy with non-disjunction in meiosis I.

In the consecutive pregnancy 2014 chorionic villi sampling (CVS) was performed due to history and high risk for trisomy 21 of first-trimester combined screening. Fetal karyotype was again 46,XX,+21,der(21;21)(q10;q10) and the pregnancy was electively terminated. However, in this case the trisomy of chromosome 21 was proved to be of the maternal origin resulting from meiosis II error. So the derivative chromosome 21 turned out to be most likely an isochromosome 21 with a corresponding karyotype description 46,XX,i(21)(q10).

Due to the plausible high recurrence risk of maternal meiotic errors the family meets the criteria for preimplantation aneuploidy screening (PGS) in prospective reproduction.

**P01.101****Prenatal diagnosis of trisomy 2 mosaicism: discrepancy between FISH study on uncultured amniocytes and karyotyping of cultured amniocytes***N. Marle<sup>1</sup>, A. Mosca-Boidron<sup>1</sup>, C. Thauvin<sup>2</sup>, T. Rousseau<sup>3</sup>, D. Lehalle<sup>2</sup>, J. Thevenon<sup>4</sup>, N. Jean-Marçais<sup>2</sup>, F. Harizay<sup>5</sup>, N. Laurent<sup>5</sup>, L. Faivre<sup>2</sup>, P. Callier<sup>1</sup>;**<sup>1</sup>Laboratoire de Cytogénétique, Plateau Technique de Biologie, CHU Dijon, Dijon, France,**<sup>2</sup>Centre de Génétique et Centre de référence „Anomalies du Développement et Syndromes Malformatifs“, Hôpital d'Enfants, CHU Dijon, Dijon, France, <sup>3</sup>Maternité du Bocage,**CHU Dijon, Dijon, France, <sup>4</sup>Centre de Génétique et Centre de référence, Dijon, France,**<sup>5</sup>Laboratoire d'Anatomo-Pathologie, Plateau Technique de Biologie, CHU Dijon, Dijon, France.*

True trisomy 2 mosaicism is rare and has been reported in only one in 58 000 cases of second-trimester amniocenteses. We report on a healthy 38-year-old G2P1 woman who underwent chorionic villus sampling (CVS) at 14 weeks of gestation because she presented an abnormal first trimester maternal serum screening (1/196). A non-mosaic 47,XX,+2 karyotype was found on direct cytogenetic analysis and on long-term CVS culture. Amniocentesis was performed at 16 weeks of gestation. FISH analysis on uncultured amniocytes revealed the trisomy 2 at a non-significant level. However, conventional cytogenetic and FISH studies on cultured amniocytes detected mosaic trisomy 2 in two independent cultures. Detailed ultrasonographic examination at 18 weeks of gestation showed an eutrophic fetus, without malformation. The patient declined the option to conduct a fetal ultrasound follow-up and decided to terminate the pregnancy.

Genetic counseling for mosaic trisomy 2 remains delicate because of the rarity of published cases and the difficulty to determine if the trisomy involves fetal tissues or only the amniotic epithelium. Moreover, it has been established that presence of autosomal trisomy in amnion correlates with poor outcome even when the fetus appears to carry only diploid cells.

To date, 19 cases of mosaic trisomy 2 detected by amniocentesis have been reported. Of these, 10 cases were associated with prominent phenotypic abnormalities. There were only four births with a normal phenotype.

Here, we conduct an exhaustive analysis of all published observations plus the present case in search of elements with prognostic value that may facilitate genetic counseling.

**P01.102****Mosaicism of trisomy 21 due to an isochromosome 21 with the normal cell line resulting from a two-step rescue***B. S. Kristiansen<sup>1</sup>, J. R. Fagerberg<sup>2</sup>, C. R. Fagerberg<sup>1</sup>;**<sup>1</sup>Odense University Hospital, Dept. of Clinical Genetics, Odense, Denmark, <sup>2</sup>Odense Katedralskole, Odense, Denmark.*

Introduction: In a pregnancy with NT of 6,7mm and maternal serum markers indicating high risk of aneuploidy, the aneuploidy screening with QF-PCR on chorion villus sample (CVS) showed trisomy 21 and chromosomal analysis showed isochromosome 21 to be the cause. However, in the aborted fetus, QF-PCR seemed compatible with normal quantities of chromosome 21.

We present how additional QF-PCR analysis reveals the structural anomaly. Materials and Methods: CVS, different tissue samples from the fetus, and blood samples from the parents.

Rapid aneuploidy screening with quantitative fluorescence PCR (QF-PCR). (Devyser Compact kit)

Standard chromosomal analysis

Results: Trisomy 21 based on an isochromosome 21 was found in CVS. QF-PCR analysis of DNA from different tissues from the aborted fetus showed

normal or borderline normal results by QF-PCR, the pattern of markers being slightly different between different tissues. Chromosomal analysis was not available for the aborted fetus. The parents had normal karyotypes. The pattern of markers in the QF-PCR-analyses was compatible with mosaicism of trisomy 21, the normal cell line resulting from a two-step rescue with loss of the maternally derived isochromosome 21 and doubling of the paternal chromosome 21. The ratio between the two cell lines in different tissues was calculated.

Conclusions: In a fetus with trisomy 21 mosaicism, QF-PCR results could wrongly be interpreted as normal. We show that the QF-PCR results reflected mosaicism of a cell line with a maternally derived isochromosome 21 and a normal cell line resulting from a two-step rescue of the trisomy 21.

#### P01.103

##### Detection aneuploidies in trophoblast cells in the model experiment

**E. Musatova, A. Martynov, Z. Markova, N. Shilova;**  
Federal State Budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation.

Introduction: Non-invasive prenatal testing implies obtaining information about the genetic status of developing fetus without damage of the fetal membranes. Trophoblast cells circulate in blood of pregnant women. It should be noted, that trophoblasts, in contrast with other fetal cells in maternal blood, are epithelial cells that are characterized by large size and distinctive morphology. For assessment opportunities of trophoblasts' isolation from the peripheral blood of pregnant women followed by analysis of these cells the model experiment was performed.

Materials and methods: There were used 11 samples of artificially created mixtures. The artificial mixtures were prepared by mixing samples of peripheral venous blood of adult with cells' samples of chorionic villus with known karyotype. There were selected two artificial mixtures for followed analysis of genetic material: for preparation these samples were used chorionic cells with karyotypes 47,XX,+13, and 47,XY,+21. There was used filtration through polycarbonate filters with a pore diameter of 8  $\mu$ m to isolate trophoblasts from artificial mixtures. Detection of trophoblasts on the filters was carried out by immunocytochemical staining with monoclonal antibodies to the cytoplasmic protein cytokeratin 7. Isolation of single cytokeratin7-positive cells was performed by laser microdissection followed by a whole genome amplification step. Analysis of genetic material isolated cytokeratin7-positive cells was performed by comparative genomic hybridization.

Results: The profiles of hybridization of the genetic material isolated cells corresponded karyotype chorionic cells that were used for the preparation of an artificial respective mixtures.

Conclusion: The obtaining results show that isolated cells were fetal origin.

#### P01.104

##### Ethnic patterns of Y chromosome microdeletions in Iranian infertile men with azoospermia/severe oligospermia

**F. Asadi, M. Sedighi Gilani, A. Ghaheri, F. Reihani-Sabet, J. Roodgar Saffari, M. Zamani;**  
Royan Institute, Tehran, Iran, Islamic Republic of

Introduction: Y chromosome microdeletion (Yq) is the most common molecular genetic cause for severe male infertility and covers three regions of AZFa, AZFb and AZFc, each of which contain various genes involved in spermatogenesis. Ethnic pattern of Yq microdeletions has not been widely studied in Iranian population. The present study aimed to reveal these patterns in Iranian infertile men with azoospermia/severe oligospermia.

Materials and Methods: 3636 infertile men referred to Royan institute with azoospermia/severe oligospermia were examined for Yq microdeletions in using multiplex PCR and different STS markers. The patients were categorized into ethnic groups based on their origin of birth and spoken language in three male generations namely Azeri, Fars, Kurd, Gilak/Mazani, Lor and Arab.

Results: Among 3636 infertile men, 177 cases of Yq microdeletions (4.8%) were diagnosed, including 60 from Azeri origin (33.8%), 55 from Fars origin (31%), 23 from Kurd origin (12.9%), 10 from Gilak/Mazani (5.6%), 18 from Lor origin (10.1%), and 2 from the Arab origin (1.1%). There were also 6 cases of non-Iranian (Afghan & Iraqi) origin. In 177 Iranian infertile men with AZF microdeletions we detected 157 microdeletions in AZFc (70%), 57 in AZFb (25.4 %) and 10 in AZFa (4.6%) regions.

Conclusion: Statistical analysis did not reveal any difference in the frequency and pattern of Yq microdeletions among various Iranian ethnic groups. Furthermore, the frequency and pattern of Yq microdeletions in Iranian population seems to be similar to the other world regions with AZFc as the most common type.

#### P02 Sensory disorders (eye, ear, pain)

##### P02.01

##### The ABCA4 c.5461-10T>C substitution in intron 38, frequently seen in Stargardt disease patients, is a splice site mutation causing skipping of exon 39-40 and reduced protein level

**I. Aukrust<sup>1</sup>, R. W. Jansson<sup>2</sup>, C. Bredrup<sup>2</sup>, S. Berland<sup>3</sup>, H. E. Rusaas<sup>1</sup>, A. Jørgensen<sup>3</sup>, M. G. Haug<sup>4</sup>, E. Rødahl<sup>2,5</sup>, G. Houge<sup>1</sup>, P. M. Knappskog<sup>1,6</sup>;**

<sup>1</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, <sup>2</sup>Department of Ophthalmology, Haukeland University Hospital, Bergen, Norway, <sup>3</sup>Division of Child and Adolescent Health, Medical Genetics Department, University Hospital of North Norway, Tromsø, Norway, <sup>4</sup>Department of Pathology and Medical Genetics, St. Olav's University Hospital, Trondheim, Norway, <sup>5</sup>Department of Clinical Medicine, University of Bergen, Bergen, Norway, <sup>6</sup>Department of Clinical Science, University of Bergen, Bergen, Norway.

Introduction: One intronic ABCA4 substitution, c.5461-10T>C in intron 38, with a minor allele frequency of 0.039% (non-Finnish, European population), is the third most common ABCA4 variant observed in patients with Stargardt disease, but its functional effect is unknown.

Materials and methods: Fibroblast samples from patients carrying the ABCA4 variant c.5461-10T>C were analyzed by isolating total RNA, followed by RT-PCR using specific primers spanning the variant. For detection of ABCA4 protein, fibroblast samples were lysed and analyzed by SDS-PAGE followed by immunoblotting using a monoclonal ABCA4 antibody.

Results: The ABCA4 variant c.5461-10T>C causes a splicing defect resulting in reduction of full-length mRNA in cells from patients and the presence of alternative spliced mRNAs where exon 39-40 is skipped. The splicing defect is causing a reduced level of full-length ABCA4 protein compared to controls not carrying the variant.

Conclusions: This study describes the functional effect and the molecular mechanism of the pathogenic ABCA4 variant c.5461-10T>C. The variant is functionally important as it leads to splicing defects and reduced level of ABCA4 protein.

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##### P02.02

##### Mutations in the amino acid transporter LAT2 (SLC7A8) cause age-related hearing loss

**M. Espino-Guarch<sup>1</sup>, E. Prat<sup>2,3</sup>, M. Font-Llitjós<sup>2</sup>, E. Errasti<sup>4</sup>, G. Girotto<sup>1</sup>, C. Vilches<sup>2</sup>, A. M. Celaya<sup>5</sup>, L. González<sup>2</sup>, D. Vuckovic<sup>1</sup>, I. Sahún<sup>6</sup>, S. Murillo-Cuesta<sup>7</sup>, M. López de Heredia<sup>8</sup>, S. Bodoy<sup>9</sup>, M. Dierssen<sup>6</sup>, I. Varela-Nieto<sup>5</sup>, P. Gasparini<sup>1</sup>, M. Palacín<sup>\*4,7</sup>, V. Nunes<sup>\*2,3</sup>, \*authors sharing seniors leadership;**

<sup>1</sup>Experimental Genetics, Sidra Medical and Research Center, Doha, Qatar, <sup>2</sup>Human Molecular Genetics Laboratory, IDIBELL, U730 Centre for Biomedical Network Research on Rare Diseases (CIBERER), L'Hospitalet de Llobregat, Barcelona, Spain, <sup>3</sup>Genetics Unit, Physiological Sciences II Department, Medicine Faculty, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain, <sup>4</sup>Institute for Research Biomedicine (IRB Barcelona), U731 Centre for Biomedical Network Research on Rare Diseases (CIBERER), Barcelona, Spain, <sup>5</sup>Neurobiology of Hearing Group, Institute for Biomedical Research "Alberto Solà" CSIC-UAM, U761 Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain, <sup>6</sup>Cellular and Systems Neurobiology, CRG, PRBB, U716 Centre for Biomedical Network Research on Rare Diseases (CIBERER), Barcelona, Spain, <sup>7</sup>Biochemistry and Molecular Biology Department, Biology Faculty, University of Barcelona, Barcelona, Spain.

Introduction: LAT2 (SLC7A8) is a neutral amino acids transporter expressed in inner ear and it has been associated with non syndromic deafness locus DFNA53. Here we demonstrate that SLC7A8 is involved in age-related hearing loss phenotype.

Materials and Methods: A null mouse model (*Slc7a8*<sup>-/-</sup>) was generated. SLC7A8 transporter has been localized in the basal layer cells of the *Stria Vascularis* and in the organ of Corti, both essential structures for proper auditory function. Auditory Brainstem Response (ABR), has been used to measure the integrity of hearing process. Human *SLC7A8* mutations screening was done in 325 individuals aged more than 50 years old. *In vitro* functional characterization of mutations found has been done overexpressing SLC7A8 mutants in HeLa cells and quantifying the alanine uptake.

Results: ABR analysis confirmed that *Slc7a8*<sup>-/-</sup> mice suffer hypoacusis earlier than C57Bl/6 old wild type mice, which suffer age-related hearing loss (ARHL). Concomitant with the phenotype observed in *Slc7a8*<sup>-/-</sup> mice, four different *SLC7A8* missense mutations had been found in ARHL patients. *In vitro* mutations functional studies demonstrated a total or partial loss of LAT2 activity.

Conclusion: Characterization of *Slc7a8*<sup>-/-</sup> mice revealed how unbalanced amino acid content in cochlea could affect auditory system and proved that LAT2 is involved in hearing mechanism. The finding of ARHL patients' carriers of *SLC7A8* mutation and the functional studies are robust evidences that

point out the significance of *SLC7A8* in auditory system. These data open new perspective where amino acid transporters could be new potential targets to unravel uncharacterized congenital deafness.

#### P02.03

#### Modifiers of gene expression as potential mechanism underlying incomplete penetrance and variable expressivity in retinal dystrophies

P. Llavona<sup>1</sup>, S. Kohl<sup>1</sup>, M. Pinelli<sup>2</sup>, M. Mutarelli<sup>2</sup>, V. Singh Marwah<sup>2</sup>, S. Schimpf-Linzenbold<sup>1</sup>, K. Nieselt<sup>1</sup>, D. di Bernardo<sup>2</sup>, S. Banfi<sup>2</sup>, B. Wissinger<sup>1</sup>

<sup>1</sup>Institute for Ophthalmic Research, Tuebingen, Germany, <sup>2</sup>TIGEM, Naples, Italy, <sup>3</sup>Center for Bioinformatics, Tuebingen, Germany.

Hereditary retinal dystrophies are frequently associated with incomplete penetrance and variable expressivity phenomena. Underlying mechanisms may include environmental, epigenetic and genetic factors with the latter being independent or linked to the primary disease causing gene such as variants involved in gene regulation. Therefore, identification of retinal disease genes that show allelic expression imbalance (AEI) in general population is a powerful approach for the identification of cis-acting expression quantitative trait loci (cis-eQTL). Our aim is to determine which cis-eQTLs could act as disease behavioral modifiers. We performed RNA-Seq from retinal RNA obtained from 52 healthy eye donors of European background. After filtering SNPs displaying extreme imbalanced allelic ratios, that are defined as lower than 0.3 and higher than 3.4 based on top 5% values, we validated heterozygosity by Sanger sequencing and AEI by pyrosequencing. Our RNA-Seq results revealed that among 301 known retinal disease genes, 64 showed evidence for AEI. *CDHR1* and *EMC1*, displaying some of the highest AEI frequencies (100% and 67% respectively), have already been positively validated by pyrosequencing. In both cases AEI is a feature exclusively present in specific gene isoforms. Comparative sequencing revealed an intronic SNP, rs4562752, strongly associated with AEI in *CDHR1-002*. *In silico* analysis predicts an impact of this variant on splice site selection and thus, explains the AEI observed for this isoform. Our results indicate that allelic transcriptional alterations caused by cis-eQTLs can indeed be traced by RNA-Seq analysis. Validation of further relevant retinal genes is still ongoing. FP7-PEOPLE-2012-ITN under grant agreement 317472.

#### P02.04

#### Deep next generation sequencing of the whole mitochondrial genome in Polish patients with aminoglycoside-induced hearing impairment

M. Rydzanicz<sup>1</sup>, A. Pollak<sup>2</sup>, U. Lechowicz<sup>2</sup>, P. Stawinski<sup>2,3</sup>, M. Wróbel<sup>3</sup>, I. Wojsyk-Banaszak<sup>4</sup>, R. Płoski<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Warsaw Medical University, Warsaw, Poland,

<sup>2</sup>Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland, <sup>3</sup>Department of Otolaryngology and Laryngological Oncology, Poznan University of Medical Sciences, Poznan, Poland, <sup>4</sup>Department of Pulmonology, Pediatric Allergy and Clinical Immunology, Poznan University of Medical Sciences, Poznan, Poland.

Introduction: Mutations in mitochondrial DNA (mtDNA) are associated with aminoglycoside-induced hearing impairment (AIHI). However, in many studies only *MTRNR1* gene is analyzed. We performed whole mtDNA sequencing for identification of known and novel AIHI mutations, mtDNA heteroplasmy assessment and haplotype prediction.

Materials and Methods: Hearing impairment patients with aminoglycoside exposure history (n=130) and normal hearing controls treated with aminoglycosides without ototoxic effect (n=100) were enrolled into the study. All participants were Caucasian. MtDNA was enriched by long-range PCR and sequenced. MToolBox was used for haplogroup assignment, functional and prioritization analysis of identified variants, and heteroplasmy fraction assessment.

Results: Mean coverage per mtDNA genome was >1000x in each subject. We found homoplasmic AIHI m.1555A>G mutation in *MTRNR1* (5/130) and several putative AIHI-related candidates, including m.7445A>G in *MTTS1* precursor (1/130, familial case) previously not associated with AIHI. Heteroplasmy analysis through the whole mtDNA genome and haplotype prediction revealed higher heteroplasmy frequency in HI patients and recurrent haplotypes not observed in control subjects (H7c - 3/130, HV10 - 2/130, R0a - 3/130, W3a - 3/130, W6a - 2/130). We also identified patient harboring low level heteroplasmy of m.3243A>G mutation in *MTTL1* associated with MELAS syndrome. This mutation was missed in previous Sanger sequencing screening and patient was misclassified to our AIHI group.

Conclusions: NGS technology is a powerful tool for mtDNA analysis in AIHI patients allowing for known and novel mutations detection. Heteroplasmy frequency and specific haplogroups observed in AIHI patients may be an additional factors modifying personal susceptibility to AIHI.

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#### P02.05

#### Microdeletions in patients with aniridia and other eye anomalies

V. I. Damyanova<sup>1</sup>, M. Mihaylova<sup>1</sup>, A. Popova<sup>2</sup>, R. Vajarova<sup>3</sup>, S. Ivanov<sup>3</sup>, L. Balabanski<sup>3</sup>, S. Hadjidekova<sup>1</sup>, R. Staneva<sup>1</sup>, D. Avdjeva-Tzavella<sup>4</sup>, R. Tincheva<sup>5</sup>, M. Malinov<sup>3</sup>, D. Toncheva<sup>1</sup>,

<sup>1</sup>Department of Medical genetics, Medical university of Sofia, Sofia, Bulgaria,

<sup>2</sup>Department of Ophthalmology, Medical University of Sofia, Sofia, Bulgaria, <sup>3</sup>Genomic laboratory, SBALGAR "Malinov", Sofia, Bulgaria, <sup>4</sup>Department of Clinical genetics-University Pediatric Hospital, Medical University of Sofia, Sofia, Bulgaria, <sup>5</sup>Department of Clinical genetics-University Pediatric Hospital, Medical University of Sofia, Sofia, Bulgaria.

Introduction: Aniridia is a rare, bilateral, congenital ocular disorder causing incomplete formation of the iris, usually characterized by iris aplasia/hypoplasia. In the majority of cases, it is caused by mutation in the *PAX6* gene, but it can also be caused by microdeletions that involve the 11p13 region. One-third of cases are sporadic and result from de novo mutations.

Materials and methods: We tested 6 patients with aniridia (full and partial forms), glaucoma and myopathy, and 1 patient with dysgenesis mesodermalis oculi with SNP microarray Illumina Human CoreExome-12.

Results: Aberrations were observed in 4 patients. Two patients with full aniridia were found to harbor heterozygous deletion in 11p13 sized 167.108 kb encompassing genes *PAX6* and *ELP4*. One patient with partial aniridia had loss of heterozygosity in part of 20p11.1p11.21 sized 1.266678 Mb, containing *VSX1* gene. *VSX1* mutations have been previously reported in patients with abnormalities of the anterior segment and keratoconus. A heterozygous deletion in 6q22.31q22.32 sized 659.704 kb, including genes: *HEY2*; *NCOA7*; *HINT3* was found in one patient with dysgenesis mesodermalis oculi. Gene *HEY2* is involved in the signal transduction pathway of *PAX6*, *FOXC1* and *PITX2* genes, associated with the disease.

Conclusion: In our study of patients with eye disorders we found in addition to a microdeletion of *PAX6*, two new deletions encompassing *HEY2* and *VSX1* genes.

#### P02.06

#### Whole exome sequencing (WES) and downstream analysis in a family with suspected "x-linked" cone rod dystrophy (CORD) reveals two disorders caused by compound heterozygote mutations in *CERKL* and *CRB1*

J. C. Gardner<sup>1</sup>, V. Plagnol<sup>2</sup>, M. Aguilà<sup>1</sup>, A. R. Webster<sup>1,3</sup>, M. Michaelides<sup>1,3</sup>, A. J. Hardcastle<sup>1</sup>,

<sup>1</sup>UCL Institute of Ophthalmology, London, United Kingdom, <sup>2</sup>UCL Genetics Institute, London, United Kingdom, <sup>3</sup>Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom.

Molecular genetic analysis of a family with apparent X-linked inheritance of CORD was performed in order to establish the cause of visual impairment. Negative mutation screening of X-linked genes *RPGR* and *RP2* in the male proband was followed by WES which revealed possible compound heterozygous mutations in two autosomal candidate genes: *CERKL* and *CRB1*.

Segregation analysis in the extended pedigree demonstrated the proband and his affected brother were compound heterozygotes for the *CERKL* variants, ENST00000410087:c.[863\_864del];[1160-1G>A] that could account for their condition, however the *CRB1* variants were both on the same (maternal) haplotype, *CRB1* :ENST00000367399:c.[exon2:493\_501del;exon4:1 024G>A]. An affected male cousin did not inherit either *CERKL* variant, but had inherited the variant *CRB1* haplotype. The cause of his condition was therefore unresolved. Sanger sequencing of the coding regions of *CRB1* in this individual did not reveal any additional mutations in other *CRB1* exons, however, cloning and sequencing of exon 2 revealed a heterozygous frameshift deletion ENST00000367399:c.[613\_619del] on the paternally derived haplotype, that had been masked by the deletion variant of the maternally derived haplotype. The phenotype in the cousin was therefore caused by compound heterozygous mutations of *CRB1*.

Our analysis has identified novel disease-causing compound heterozygous variants in *CERKL* and *CRB1* and shows two autosomal recessive retinal disorders masquerading as an X-linked condition in this pedigree, thus creating issues for genetic counselling. This illustrates the importance of segregation analysis in the extended family for the successful interpretation of WES data. This work was supported by Moorfields Special Trustees.

## P02.07

### Next generation sequencing discovers novel splice variants causing deafness in the Middle Eastern population

*N. Danial-Farran*<sup>1,2</sup>, *L. Tamer*<sup>2</sup>, *Z. Brownstein*<sup>3</sup>, *F. T. A. Martins*<sup>2</sup>, *M. Khayat*<sup>1</sup>, *G. Ast*<sup>2</sup>, *S. Allon-Shalev*<sup>1</sup>, *K. B. Avraham*<sup>2</sup>;

<sup>1</sup>Genetics Institute, Ha'Emek Medical Center, Afula, Israel and Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, <sup>2</sup>Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, <sup>3</sup>Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Afula, Israel.

The numbers of reported pathogenic variants causing genetic disorders have increased dramatically since Next Generation Sequencing has become available. The Middle Eastern population, known to exhibit high rates of consanguinity and comprised of isolated ethnic groups, has contributed dramatically to the discovery of pathogenic variants causing deafness. We applied targeted genomic enrichment (TGE), along with massively parallel sequencing (MPS), of 284 genes on unrelated Middle Eastern families. Our results doubled the number of genes associated with deafness in this population. A number of these required functional validation to determine the consequences of the variants. One of these was a synonymous alteration located in the 5' splice site of the MYO15A gene. Our challenge was to prove the consequences of this variant, since we could not check the splicing in the patient's RNA, due to the inaccessibility of human inner ear tissue and lack of MYO15A expression in lymphoblasts. As a result, a functional splicing assay was performed to determine the consequences of the variant on splicing. Due to the high GC content surrounding the splice site, we created a modified human cell line expressing the wild type and mutant forms of the genomic region. Our results indicate that the mutation abolishes intron retention, an event that is likely to be a functional alternative splicing event. Considering the importance of providing evidence for pathogenic variants, our study demonstrates the use of a robust tool for investigating splicing variants, particularly for genes expressed in inaccessible tissues.

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## P02.08

### Exon-skipping allows passing complete CEP290 loss-of-function in an individual with unusually mild retinal disease

*X. GERARD*<sup>1</sup>, *I. Perrault*<sup>1</sup>, *A. Munnich*<sup>2</sup>, *C. Hamel*<sup>3</sup>, *J. Kaplan*<sup>1</sup>, *J. Rozet*<sup>1</sup>;

<sup>1</sup>Institut Imagine - Lab of Genetics in Ophthalmology - Inserm U1163 - Université Paris Descartes, Paris, France, <sup>2</sup>Institut Imagine - Genetics department - Inserm U1163 - Université Paris Descartes, Paris, France, <sup>3</sup>Maladies Sensorielles Génétiques, CHRU - INSERM U1051, Institute for Neurosciences, Montpellier, France.

Introduction. CEP290 is pivotal in the assembly/maintenance of primary and motile cilia in a wide range of cell systems. Biallelic CEP290 mutations cause a wide spectrum of devastating ciliopathies ranging from mono-symptomatic retinal dystrophy to multi-visceral sometimes embryo-lethal syndromes. The CEP290 retinal disease manifests invariably as a congenital and dramatically severe cone-dominant disease with visual function reduced to light perception. Using targeted-NGS in individuals with retinal dystrophies, we identified a homozygote CEP290 1pb deletion (c.1666del; p.Ile 556Phefs\*17) in a 15 year-old girl having a rod dominant disease with preserved cone function (visual acuity = 6/10). The present study aimed at understanding the molecular bases of this observation.

Material and Methods. RT-PCR, RTqPCR, Western blot and cilia analyses were performed from patient and control fibroblasts.

Results. Analysis of the CEP290 mRNA in patient fibroblasts revealed low levels of an altered splice form in which exon 18 was skipped (5% of the amount of the wildtype mRNA in controls) and Western blot detected low levels of a CEP290 protein around 290 KDa. The number of patient fibroblasts producing a cilium under serum starvation was in the normal range. Conclusions. The mutation identified in the patient is predicted to encode a premature stop codon in exon 18, downstream of the 1bp deletion in exon 17. The skipping of exon 18 with the upstream 1bp deletion allows producing low levels of CEP290 mRNA with an intact open reading frame and of a minimally shortened protein which expression may explain the preservation of central vision.

## P02.09

### Unravelling human complex traits: the case of hearing function and age related hearing loss

*D. Vuckovic*<sup>1</sup>, *A. Morgan*<sup>2</sup>, *M. P. Concas*<sup>3</sup>, *M. Ciullo*<sup>4</sup>, *T. Nutile*<sup>4</sup>, *M. Pirastu*<sup>3</sup>, *M. J. Huentelman*<sup>5</sup>, *I. Schrauwen*<sup>5</sup>, *R. A. Friedman*<sup>6</sup>, *E. Franssen*<sup>7</sup>, *G. Van Camp*<sup>7</sup>, *P. Gasparini*<sup>1,2</sup>, *G. Girotto*<sup>2,1</sup>;

<sup>1</sup>Sidra Medical and Research Centre, Doha, Qatar, <sup>2</sup>University of Trieste, Trieste, Italy,

<sup>3</sup>Institute of Population Genetics, CNR, Sassari, Italy, <sup>4</sup>Institute of Genetics and Biophysics, A. Buzzati-Traverso, CNR, Naples, Italy, <sup>5</sup>Translational Genomics Research Institute,

Phoenix, AZ, United States, <sup>6</sup>Keck Medicine of USC, Los Angeles, CA, United States,

<sup>7</sup>University of Antwerp, Antwerp, Belgium.

In order to overcome the lack of knowledge on the molecular bases of complex hearing traits (Normal Hearing Function-NHF, Age-Related Hearing Loss-ARHL), a fruitful gene discovery strategy is proposed: new candidate genes/variants identified through GWAS/metanalysis are then validated by targeted re-sequencing (TRS) and functional studies in animal models.

GWAS on NHF (quantitative) and ARHL (qualitative) on 4150 subjects from different cohorts (Italian isolates, Belgium, Caucasus and Central Asia) were performed. Two strongly significant loci (p-value~2e-8) were identified, one of which (top-SNP:rs11520167) includes a gene causing hearing loss in mice and affecting cochlear outer hair cells. Interestingly, it is the first example of a hearing-loss gene modulating NHF.

To further investigate the role of candidates identified we developed a custom TRS panel of 46 genes and analysed ~500 ARHL patients from our cohorts. 56 mutations of interest were identified: 5 frameshift indels, 2 nonsense and 49 missense affecting 22 different genes and 128 patients. According to the complex genetic structure of ARHL, three scenarios were detected: 1) different mutations in the same gene, 2) the same mutation in different patients and 3) different mutations in the same patient. Finally genes were prioritised and included for expression analysis in the otic vesicle of Zebrafish larvae and for the generation of Knock-Out/In models and their accurate phenotypic characterisation. Results from the first series of genes (e.g. SLC44A2 and SLC9A3R1) will be presented and discussed. These results strongly support the usefulness of our approach to detect and validate new genes involved in NHF and ARHL.

## P02.10

### Evaluation of a progressive diagnostic strategy to identify the genetic etiology of hearing loss

*J. S. Crettaz*<sup>1</sup>, *R. Muguerza*<sup>1</sup>, *L. Otaolea*<sup>1</sup>, *Z. Martinez*<sup>2</sup>, *M. Abrego*<sup>2</sup>, *Y. Ramirez*<sup>1</sup>, *R. Saez*<sup>1</sup>;

<sup>1</sup>Genetics Department, Donostia University Hospital, San Sebastian, Spain,

<sup>2</sup>Otorhinolaryngology Department, Donostia University Hospital, San Sebastian, Spain.

Introduction: Hearing loss is the most prevalent sensory deficit and is caused by genetic, environmental or multifactorial etiologies. It traditionally presented a challenge for genetic testing due to its heterogeneity.

Materials and Methods: Seventy-one patients with early-onset sensorineural hearing loss were enrolled. First Tier approach includes the study of m.1555 and m.1494 mitochondrial mutations and MLPA study for identification of duplications/deletions and common point mutations in GJB2 and GJB6 genes. Second Tier was performed using a custom NGS panel of 92 genes on 16 negative patients. Haloplex technology (Agilent Technologies) was used for exons and flanking intronic sequences enrichment and sequencing was performed on a MiSeq (Illumina).

Results: First-tier strategy detected 1 homoplasmic m.1555 mutation in MT-RNR1 gene (1,5%) and 8 pathogenic conditions in GJB2 and GJB6 genes (12%). Second-tier produced a coverage of targeted exons for >20 reads of 96,3% and a mean depth coverage of 454x. By our filtering strategy, the patients were classified as follow: 5 resulted with a pathogenic variant (31%), 5 with uncertain variants (31%) and 6 with irrelevant findings (38%). All relevant variants were confirmed by Sanger sequencing and characterization of novel variants is in course.

Conclusions: Diagnostic capacity of NGS is indubitable although the progressive approach presented here may be useful to maintain a cost-effective strategy and to cover all the technically excluding needs (mitochondrial, large copy number variants, single nucleotide variants). Preliminary results of our testing strategy reached an overall diagnostic yield of 44%.

## P02.11

### Distribution of pathogenic variants in non-SLC26A4/PDS hearing impaired patients with inner ear abnormalities: initial results from a Czech cohort

*R. Pourová*<sup>1</sup>, *J. Paděrová*<sup>1</sup>, *J. Sabová*<sup>1</sup>, *R. Katra*<sup>2</sup>, *M. Jurovčík*<sup>2</sup>, *M. Havlovicová*<sup>1</sup>, *M. Macek, jr.*<sup>1</sup>;

<sup>1</sup>Department of Biology and Medical Genetics, University Hospital Motol, Prague, Czech Republic, <sup>2</sup>Department of ENT, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Prague, Czech Republic.

Hereditary hearing loss (HHL) is the most common inborn sensory deficit affecting about 1 in 300 neonates with a strong genetic aetiology (up to 75%) and is mainly associated with AR- and non-syndromic pattern inheritance. However, de novo AD pathogenic variants may also be found, while syndromic features could be rather mild or even misleading during childhood.

Five families from the cohort of SLC26A4/PDS negative patients with HHL

compounded by inner ear abnormalities (IEA) were analysed. One family has AR, another AD trait; in three cases the occurrence of HHL was apparently sporadic. Four cases did not have syndromic signs, while one case had a syndromic form of HHL.

Extended gene panel next generation sequencing (NGS) was carried out in all studied cases (TruSight One, Illumina, USA), verified by Sanger DNA sequencing in detected variants.

We have identified likely pathogenic variants in all examined families. Most commonly compound heterozygous pathogenic variants were found in MYO15A (2 families) associated with AR NSHL, while compound heterozygous MYO7A variants linked to Usher syndrome 1B and de novo AD EYA1 and CHD7 variants causing BOR- and CHARGE syndromes respectively, were observed.

In conclusion, this pilot study provided evidence that the genetic aetiology of inner ear disorders is heterogeneous. Although BOR- and CHARGE syndromes are well documented causes of HHL with IEA, pathogenic variants in MYO15A and MYO7A have not been reported, thus far. Further studies utilising NGS are thus warranted.

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#### P02.13

##### Distinct clinical and radiological phenotype associated with POU3F4 mutations

**A. Pollak<sup>1</sup>, U. Lechowicz<sup>1</sup>, A. Kędra<sup>2,3</sup>, P. Stawiński<sup>1</sup>, M. Rydzanicz<sup>2</sup>, M. Mrówka<sup>4</sup>, P. Skarżyński<sup>5,6</sup>, M. Furmanek<sup>7,8</sup>, H. Skarżyński<sup>9</sup>, R. Płoski<sup>2</sup>, M. Oldak<sup>1</sup>;**

<sup>1</sup>Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland, <sup>2</sup>Department of Medical Genetics, Warsaw Medical University, Warsaw, Poland, <sup>3</sup>Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland, <sup>4</sup>Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland, <sup>5</sup>Institute of Physiology and Pathology of Hearing, Warsaw, Poland, <sup>6</sup>Department of Heart Failure and Cardiac Rehabilitation, Medical University of Warsaw, Warsaw, Poland, <sup>7</sup>Bioimaging Research Center, World Hearing Centre of the Institute of Physiology and Pathology of Hearing, Warsaw/Kajetany, Poland, <sup>8</sup>Department of Radiology and Diagnostic Imaging, Medical Centre for Postgraduate Education, Warsaw, Poland.

POU3F4 mutations (DFNX) are the most prevalent cause of hearing loss among all the X-linked loci. The aim of the study was to screen for POU3F4 mutations in a large group of hearing loss patients, analyze audiological and radiological features in patients with HL caused by POU3F4 defects and to present our experience on cochlear implantation in these patients. Mutations in the POU3F4 gene were first analyzed in a selected group of patients with gusher or gusher-like incidence (n= 26) through sequencing of the whole gene. Subsequently, the identified mutations were screened for in a cohort of two thousand males with HL. The molecular techniques used to detect POU3F4 mutations included whole exome sequencing, Sanger sequencing and real-time PCR. Three novel (p.Glu187\*, p.Leu217\*, p.Gln275\*) and one known p.Ala116fs141\* POU3F4 mutations were detected. One of the novel mutations was revealed to be a de novo event. All probands with POU3F4 defects suffered from bilateral prelingual severe to profound HL. Morphological changes of the temporal bone in these patients presented a similar pattern, including malformations of the internal auditory canal, vestibular aqueduct, modiolus and vestibule. Two of the patients with POU3F4 mutations received cochlear implants with good outcome. In summary, extensive clinical and genetic investigations show that POU3F4 mutations frequently occur in patients with particular temporal bone malformations. In this group of patients sequencing of the entire POU3F4 gene is necessary due to specific requirements and an increased risk of complications during otologic surgery.

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#### P02.14

##### Molecular findings from 537 individuals with inherited retinal disease

**J. M. Ellingford<sup>1</sup>, S. Barton<sup>2</sup>, S. Bhaskar<sup>2</sup>, J. O'Sullivan<sup>1</sup>, S. G. Williams<sup>1</sup>, J. A. Lamb<sup>1</sup>, B. Panda<sup>3</sup>, P. I. Sergouniotis<sup>1</sup>, R. L. Gillespie<sup>1</sup>, S. P. Daiger<sup>4</sup>, G. Hall<sup>2</sup>, T. Gale<sup>2</sup>, C. Lloyd<sup>1</sup>, P. N. Bishop<sup>1</sup>, S. C. Ramsden<sup>1</sup>, G. C. M. Black<sup>1</sup>;**

<sup>1</sup>University of Manchester, Manchester, United Kingdom, <sup>2</sup>Central Manchester University NHS Foundation Trust, Manchester, United Kingdom, <sup>3</sup>GANIT Laboratories, Institute for Bioinformatics and Applied Biotechnology, Bangalore, India, <sup>4</sup>University of Texas, Texas, TX, United States.

Background: Inherited retinal diseases (IRD) are a clinically and genetically heterogeneous set of disorders, for which diagnostic next-generation sequencing (NGS) services have been developed worldwide.

Methods: We present the molecular findings for 537 individuals referred to

a 105-gene diagnostic NGS test for IRD. We assess the diagnostic yield, the spectrum of clinical referrals, the variant analysis burden, and the genetic heterogeneity of IRD. We retrospectively analyze disease-causing variants, including an assessment of variant frequency in ExAC.

Findings: Individuals were referred from 10 clinically distinct classifications of IRD. Of the 4,542 variants clinically analysed, we have reported 402 mutations as a cause or a potential cause of disease in 62 of the 105 genes surveyed. These variants account or likely account for the clinical diagnosis of IRD in 51% of the 537 referred individuals. 144 potentially disease-causing mutations were identified as novel at the time of clinical analysis, demonstrating a clear advantage of diagnostic NGS approaches for IRD but also highlighting the need for further evidence to assist clinical interpretation. Only 15% of molecular diagnoses are accounted for by mutations in the 33 genes identified as a cause of IRD post-2005, suggesting that whilst the continued addition of new disease-causing genes will of course improve diagnostic services, there is a need to survey intronic and regulatory regions of genes known as a cause of IRD.

Interpretation: Our findings illustrate the continued powerful utility of custom-gene panel diagnostic NGS tests for IRD in the clinic, but suggest clear future avenues which will increase diagnostic yields.

#### P02.15

##### Whole genome sequencing findings in Spanish families affected with Retinal Dystrophies

**R. Sanchez-Alcudia<sup>1</sup>, N. Bedoni<sup>2</sup>, B. Royer-Bertrand<sup>2</sup>, M. Corton<sup>1</sup>, A. Avila-Fernandez<sup>1</sup>, R. Perez-Carrio<sup>1</sup>, N. Sanchez-Bolivar<sup>1</sup>, O. Zurita<sup>1</sup>, K. Nikopoulos<sup>2</sup>, S. Balzano<sup>2</sup>, C. Rivolta<sup>2</sup>, C. Ayuso<sup>1</sup>;**

<sup>1</sup>Department of Genetics, IIS-Fundacion Jimenez Diaz University Hospital, Madrid, Spain, <sup>2</sup>Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland.

To study the genetic basis and to identify pathogenic mechanisms of Retinal Dystrophies (RD), a total of 10 Spanish families, remaining uncharacterized by Whole Exome Sequencing (WES), were analyzed by Whole Genome Sequencing (WGS). The WGS data were processed following an expert in-house in silico pipeline developed by the University of Lausanne. We were able to characterize 4 out of 10 (40%) of the families. Biallelic single nucleotide variations (SNV), undetected by WES due to the low-depth coverage of the specific region, were detected in an X-linked Retinitis Pigmentosa and two autosomal recessive RD families with mutations in the NYX, DFNB31 and ACBD5 genes, respectively. The identification of a nonsense mutation in the NYX gene, responsible for congenital stationary night blindness, led to the reassessment of the clinical diagnosis. Interestingly, the WGS analytical pipeline in combination with homozygosity mapping analysis were able to identify a novel homozygous mutation in the ACBD5 gene in a maternal isodisomy case, leading to a new phenotype-genotype association. A combination of a non biallelic SNV and a structural variation was found in an additional arRD family. Affected members of this family were compound heterozygous for a missense variant and a ~56kb deletion involving exons 32 and 33 in the EYS gene. In this study, we show the power of WGS coupled with homozygosity mapping in solving recessive cases. Moreover, we demonstrate not only that WGS allows the detection of structural events, but it also has a more uniform and reliable sequencing coverage compared to WES.

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#### P02.16

##### Improving the management of Inherited Retinal Dystrophies by targeted sequencing of a population-specific gene panel

**C. Mendez-Vidal<sup>1,2</sup>, N. Bravo-Gil<sup>1,2</sup>, L. Romero-Perez<sup>1</sup>, M. Gonzalez-del Pozo<sup>1,2</sup>, E. Rodriguez-de la Rua<sup>3</sup>, J. Dopazo<sup>4,5</sup>, S. Borrego<sup>1,2</sup>, G. Antón<sup>1,2</sup>;**

<sup>1</sup>Institute of Biomedicine of Seville, University Hospital Virgen del Rocío/CSIC/University of Seville, Sevilla, Spain, <sup>2</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Sevilla, Spain, <sup>3</sup>University Hospital Virgen Macarena, Sevilla, Spain, <sup>4</sup>Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain, <sup>5</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Valencia, Spain.

Introduction: Molecular diagnosis of Inherited Retinal Dystrophies (IRD) has long been challenging due to their extensive clinical and genetic heterogeneity. Here, we describe an efficient next generation sequencing (NGS)-based diagnostic tool for the identification of causative mutations in a Spanish cohort with diverse IRD.

Methods: We used a custom NGS panel comprising 64 IRD-associated genes in our population, and three disease-associated intronic regions for the molecular diagnosis of 32 families with a wide range of IRD. Targeted bases were captured and sequenced on the Illumina MiSeq platform. Subsequently, bioinformatics and co-segregation analyses were performed to identify causative variants.

**Results:** A total of 37 pathogenic mutations (14 novel) were found in 73% ofIRD patients ranging from 50% for autosomal dominant cases, 75% for syndromic cases, 83% for autosomal recessive cases, and 100% for X-linked cases. Additionally, unexpected phenotype-genotype correlations were found in 6 probands, which led to the refinement of their clinical diagnoses. Furthermore, intra- and interfamilial phenotypic variability was observed. Finally, two cases unsuccessfully analysed by exome sequencing were resolved by applying this panel.

**Conclusions:** Our results demonstrate that this hypothesis-free approach based on frequently mutated, population-specific loci is highly cost-efficient for the routine diagnosis of this heterogeneous condition and allows the unbiased analysis of a miscellaneous cohort. The molecular information found here has aid clinical diagnosis and has improved genetic counselling and patient management.

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#### P02.17

#### Unraveling the genetic basis of simplex cases of Retinitis Pigmentosa

*N. Bravo-Gil<sup>1,2</sup>, M. González-del Pozo<sup>1,2</sup>, C. Méndez-Vidal<sup>1,2</sup>, M. Martín-Sánchez<sup>1</sup>, S. Borrego<sup>1,2</sup>, G. António<sup>1,2</sup>;*

<sup>1</sup>Department of Genetics, Reproduction and Fetal Medicine, Institute of Biomedicine of Seville, University Hospital Virgen del Rocío/CSIC/University of Seville, Seville, Spain,

<sup>2</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Seville, Spain.

**Introduction:** The purpose of this study was to elucidate the genetic cause of the disease in Spanish patients with simplex (isolated) Retinitis Pigmentosa (RP), allowing to determine the proportion of these cases with autosomal recessive, *de novo* autosomal dominant or X-linked RP.

**Materials and Methods:** A capture panel containing 68 genes associated with Inherited Retinal Dystrophies (IRD) in Spanish population was used for the molecular diagnosis of 24 simplex cases of RP. Targeted bases were sequenced on the Illumina MiSeq platform.

**Results:** Gene panel sequencing generated a mean coverage of 915x and a mean of reads on target of 96.98%. A total of 19 pathogenic mutations, including point mutations, frameshift mutations and large deletions, were found in 13 out of 24 patients, obtaining a diagnostic rate of 54.17%. Of families solved, 69.2% were autosomal recessive cases, 15.4% carried mutations in X-linked IRD genes and 15.4% were autosomal dominant cases with *de novo* mutations.

**Conclusions:** Our results indicate that the most common pattern of inheritance of simplex cases of RP is autosomal recessive but, due to family impact, other options should not be overlooked. To our knowledge, this is the first study to apply next-generation sequencing to seek for causative mutations in Spanish simplex RP cases, deciphering the prevalence of the different modes of inheritance behind these types of pedigrees and improving the subsequent clinical management of patients and respective families.

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#### P02.18

#### Panel-based NGS reveals: a new mutation in the LTBP2 gene causing Isolated Ectopia Lentis in a Spanish consanguineous family

*S. Bernal<sup>1</sup>, J. Crespi<sup>2</sup>, L. Alías<sup>1,3</sup>, L. González-Quereda<sup>1,3</sup>, P. Gallardo<sup>1,3</sup>, M. Baiget<sup>3</sup>;*

<sup>1</sup>Genetics Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,

<sup>2</sup>Ophthalmology Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,

<sup>3</sup>U705-CIBERER, Barcelona, Spain.

**Introduction:** The ectopia lentis is a rare disease characterized by the presence of crystalline bilaterally smaller and more spherical than the normal size (microspherophakia). The lens subluxation can lead refractive error, glaucoma due to pupillary block and vision loss. Although it is generally associated with systemic disorders (Marfan, Weill-Marchesani syndromes or homocystinuria), it is also described an isolated form (IEL) showing an autosomal recessive inheritance, and characterized by a high phenotypic variability and genetic heterogeneity (ADAMTSL4, FBN1, LTBP2, ADAMTSL10 and ADAMTSL17). **Material:** All members of a Spanish consanguineous family were studied, of which two sons were affected by IEL without megalocornea. **Methods:** 1) Next generation Sequencing (NGS) by TruSight One panels (Illumina) of the patient samples. 2) Validation of the NGS results by Sanger sequencing method. 3) Confirmation of a common origin of the detected mutation in the LTBP2 gene using the polymorphic markers D14S43 and D14S999. **Results:** Several genetic variants were identified by NGS, only one showed a pathogenic effect. This mutation involves the insertion of an ade-

nine in the exon 36 of the LTBP2 gene (c.5439\_5440insA). **Familial study** by the polymorphic markers confirmed a common haplotype associated with this mutation previously not described. **Conclusion:** The LTBP2 gene has been described in non-European families as a responsible of IEL associated with megalocornea or congenital glaucoma (OMIM#251750). Panel-based NGS approach has allowed the identification of a new mutation in the LTBP2 gene, which confers a different phenotypic variation to IEL: no megalocornea or congenital glaucoma.

#### P02.19

#### Variants in SKP1, PROB1, and IL17B associated with familial keratoconus identified by linkage analysis combined with whole exome sequencing

*J. A. Karolak<sup>1,2</sup>, T. Gambin<sup>3,4,5</sup>, J. A. Pitarque<sup>6</sup>, A. Molinari<sup>6</sup>, S. Jhangiani<sup>4</sup>, P. Stankiewicz<sup>4</sup>, J. R. Lupski<sup>7,8</sup>, M. Gajecka<sup>2,1</sup>;*

<sup>1</sup>Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Poznan, Poland, <sup>2</sup>Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, <sup>3</sup>Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland, <sup>4</sup>Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>5</sup>Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland, <sup>6</sup>Department of Ophthalmology, Hospital Metropolitano, Quito, Ecuador, <sup>7</sup>Department of Molecular & Human Genetics; Human Genome Sequencing Center; Department of Pediatrics, Baylor College of Medicine, Houston, TX, United States, <sup>8</sup>Texas Children's Hospital, Houston, TX, United States.

Keratoconus (KTCN) is a protrusion and thinning of the cornea, resulting in impairment of visual function. The extreme genetic heterogeneity makes it challenging to identify factors unambiguously influencing the KTCN phenotype. To identify a potential genetic susceptibility component which may be involved in familial KTCN, we used linkage analysis, whole exome sequencing (WES), and Sanger sequencing in an Ecuadorian family with KTCN. We applied WES in two affected KTCN individuals from the family that showed a suggestive linkage between the KTCN phenotype and the 5q31.1-q35.3 locus. Putative rare variants identified in WES analysis were confirmed and further evaluated for segregation in this family using Sanger sequencing. Exome capture discovered a total of 173 rare (MAF <1% in control population) nonsynonymous variants in both affected individuals. Among them, 16 SNVs were selected for further study. Segregation analysis revealed that variants c.475T>G (p.Cys159Gly) in *SKP1*, c.671G>A (p.Gly224Asp) in *PROB1*, and c.527G>A (p.Cys176Tyr) in *IL17B* in the 5q31.1-q35.3 linkage region co-segregated with the phenotype. We demonstrate that a combination of linkage analysis, WES, and Sanger sequencing, significantly narrowed reduced the list of the putative variants. Moreover, since the analyzed locus overlapped two other chromosomal regions previously recognized in distinct KTCN studies, our findings further suggest that this 5q31.1-q35.3 locus might be linked with KTCN.

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#### P02.20

#### A targeted-resequencing gene panel for the genetic diagnosis of Leber congenital amaurosis

*M. Plumar<sup>1</sup>, A. Asaro<sup>1</sup>, S. Zucca<sup>1</sup>, M. Valente<sup>1</sup>, G. S. Grieco<sup>1</sup>, S. Signorini<sup>2</sup>, C. Cereda<sup>1</sup>;*

<sup>1</sup>Center of Genomics and post-Genomics, C. Mondino National Neurological Institute, Pavia, Italy, <sup>2</sup>Child Neurology and Psychiatry Unit, C. Mondino National Neurological Institute, Pavia, Italy.

**Introduction:** Leber congenital amaurosis (LCA) is a group of early-onset childhood retinal dystrophies characterized by vision loss, nystagmus and severe retinal dysfunction. Currently, mutations in over 20 genes have been reported to cause LCA, mostly with an autosomal recessive pattern of inheritance, although a genetic diagnosis is hard to achieve because of the genetic heterogeneity. The aim of this study was to obtain a fast and accurate strategy for genetic diagnosis of LCA.

**Materials and Methods:** coding regions of 24 genes implicated in LCA were analyzed in a cohort of 27 unrelated patients referred to our laboratory, by performing a TruSeq Custom Amplicon (Illumina) assay for targeted-resequencing.

**Results:** two and one mutations were detected in 16 and 6 patients, respectively. Sanger sequencing of uncovered regions identified further mutations in 4 patients. The segregation analysis was performed on the available parents. Twenty cases were fully diagnosed: 7 homozygotes and 12 compound heterozygotes (recessive genes), and one heterozygous subject (dominant genes). Overall, 32 different pathogenetic variants were identified (half of which were new mutations); nonsense, missense, frameshift and

splicing mutations were found mostly in 6 genes (AIPL1, CEP290, GUCY2D, MNAT1, RPGRIP1, SPATA7).

Conclusions: in a high percentage of confidently diagnosed LCA patients, mutations may be found in known recessive genes; our targeted-resequencing gene panel allows to detect a high number of described and new mutations, turning out to be a valid method for genetic diagnosis of LCA.

## P02.21

### Genetic heterogeneity in autosomal dominant familial Meniere Disease

C. Martín-Sierra<sup>1</sup>, T. Requena<sup>1</sup>, A. Gallego-Martinez<sup>1</sup>, A. Lysakowski<sup>2</sup>, J. Lopez-Escamez<sup>1</sup>, <sup>1</sup>GENYO, Granada, Spain, <sup>2</sup>Dept. of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL, United States.

Introduction: Meniere's Disease (MD) is a complex disorder characterized by recurrent attacks of spontaneous vertigo associated with sensorineural hearing loss and tinnitus, with a strong familial aggregation (prevalence of 8-10% of familial cases). We have performed whole-exome sequencing (WES) analysis in three different families with autosomal dominant inheritance pattern of MD, to study the genetic causes of this disorder; identifying the candidate variants segregating the MD phenotype.

Materials and Methods: WES data were processed in SOLiD 5500xl platform. We filtered the Single Nucleotide Variants (SNVs) by dbSNP 142, ExAC databases and our in house control database, using a minor allelic frequency MAF > 0.0001. To prioritize candidate variants we used the following bioinformatics tools: Pathogenic Variant –PAVAR score, an in house scoring system based upon seven tools (SIFT, PolyPhen2, Grantham's Matrix, GERP+, Mutation taster, PhastCons and PhyloP) estimating the effect in protein structure and phylogenetic conservation; and bioinformatics tools that include phenotype information such as Exomiser v2 and Variant Annotation Analysis and Search Tool (VAAST) + Phevor. All variants were validated by Sanger sequencing technology in a 3130 Genetic Analyzer.

Results: After filtering and prioritizing, we have generated a list of candidate pathogenic SNVs, and we confirmed the expression of these genes in inner ear human tissue. The top candidate genes are PRKCB, SEMA3D and DPT.

Conclusion: Our results support genetic heterogeneity in familial MD.

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## P02.22

### WES and mutation analysis in 26 families reveal a new founder MYO15A frameshift duplication as the major cause of Genetic Hearing Loss in Oman

F. Palombo<sup>1</sup>, T. Pippucci<sup>1</sup>, N. Al-Wardy<sup>2</sup>, M. Oppo<sup>3,4</sup>, M. Al Kindi<sup>2</sup>, A. Angius<sup>3,5</sup>, K. Al Lamki<sup>2</sup>, G. A. Gnechi Ruscone<sup>6</sup>, T. Giangregorio<sup>1</sup>, M. Benelli<sup>7</sup>, F. Cucca<sup>3,4</sup>, A. Magri<sup>8</sup>, M. Seri<sup>1</sup>, M. Sazzini<sup>9</sup>, M. Al Khabori<sup>9</sup>, G. Romeo<sup>1</sup>;

<sup>1</sup>Medical Genetics Unit, Polyclinic Sant'Orsola-Malpighi, Bologna, Italy, <sup>2</sup>College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman, <sup>3</sup>Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Cagliari, Italy, <sup>4</sup>Dipartimento di Scienze Biomediche, University of Sassari, Sassari, Italy, <sup>5</sup>Center for Advanced Studies, Research, and Development in Sardinia (CRS4), Parco Scientifico e Tecnologico della Sardegna, Pula, Italy, <sup>6</sup>Laboratory of Molecular Anthropology, University of Bologna, Bologna, Italy, <sup>7</sup>Department Laboratory, Careggi University Hospital, Firenze, Italy, <sup>8</sup>Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy, <sup>9</sup>Department of ENT, Al Nahda Hospital, Ministry of Health, Muscat, Italy.

The prevalence of Genetic Hearing Loss (GHL) in Oman is estimated to be 6/10000 and mutations in GJB2 are known to be absent; however a thorough molecular genetic assessment of GHL in Oman is lacking. Families of children with GHL have high consanguinity rates, indicating a major role for autosomal recessive forms. We recruited 43 GHL patients from 26 North Omani consanguineous families and analyzed one of these by Whole Exome Sequencing (WES), identifying a novel homozygous frameshift duplication (c.1171\_1177dupGCCATCT) in MYO15A, a gene associated with autosomal recessive deafness (DFNB3). This was then found in 8/26 (28%) families, in heterozygous state in 2/190 subjects from North Oman and absent in 94 subjects from South Oman. The duplication was within a 849 Kb founder haplotype and using SNPs located on a 1Mb genomic interval including MYO15A to infer haplotypes in disease individuals and in around 2,500 healthy worldwide subjects we reconstructed a median joining network to get insights into the origins and evolutionary history of the MYO15A duplication. We then performed WES in 12 families out of the 18 non MYO15A-mutated and identified mutations in different genes which are likely to be causative of GHL in 7/12 families with an overall diagnostic rate of 58% (15/26). In conclusion, the MYO15A duplication, found in 28% of families and with a carrier population frequency of 1%, emerges as the major cause

of GHL in North Oman and the strategy described could have primary implications for an efficient design of GHL diagnosis in Oman.

## P02.23

### Targeted/Whole Exome Sequencing plus Animal Models for the molecular characterisation of Hereditary Hearing Loss (HHL)

A. Morgan<sup>1</sup>, D. Vozzi<sup>2</sup>, E. Rubinato<sup>1</sup>, M. Khalifa Al-Kowari<sup>2</sup>, M. Di Stazio<sup>1</sup>, R. Badii<sup>3</sup>, K. Abdulkhadi<sup>3</sup>, M. La Bianca<sup>4</sup>, P. Gasparini<sup>1</sup>, G. Girotto<sup>1</sup>;

<sup>1</sup>University of Trieste, Trieste, Italy, <sup>2</sup>Sidra medical research, Doha, Qatar, <sup>3</sup>Hamad Medical Corporation, Doha, Qatar, <sup>4</sup>IRCCS Burlo Garofolo, Trieste, Italy.

HHL is characterised by clinical and genetic heterogeneity. Here, 70 Italian and Qatari HHL families were screened by analysis of 113 HHL-genes by Targeted Re-Sequencing (TRS) followed, in negative cases, by Whole Exome Sequencing (WES) to discover HHL-candidate genes and by in vitro and in vivo validation of them.

Ion Torrent PGM™ (4.356 amplicons ensuring approximately 92,6% coverage of the target region) and Ion Proton™ (293.903 amplicons ensuring approximately >99% coverage of the target region) were used for TRS and WES, respectively. Genomic variants were annotated by ANNOVAR and filtered according to: a) pedigree pattern of inheritance, b) allele frequency data, c) pathogenicity prediction. Functional studies were carried out in Zebrafish (expression and generation of K/O-K/J).

TRS allowed us to characterise 9/25 Italian and 8/15 Qatari families (overall detection rate of 43%) while for other 30 TRS-studies are in progress.

As regards to WES, 19 cases entered the pipeline. Some new genes (i.e. BDP1; PSIP1) and several strong candidates (e.g. TBL1Y, PIEZO1, LAMC1, PLS1) for which final functional validation is in progress were identified. Preliminary data include: 1) expression studies for PLS1 in Zebrafish larvae revealing a strong gene-expression in the hair cells of the otic vesicle; 2) RT-PCR on TBL1Y showing a cochlear expression in the Human cDNA and transfection of HEK cells displayed that the mutation alters the stability of the protein itself. These findings highlight the relevance of the combined approach based on NGS and animal model validation in understanding the genetics of HHL. Updated data will be presented and discussed.

## P02.24

### Targeted Next Generation Sequencing for Diagnosis of Patients with Congenital Eye Malformations

M. Tarilonte<sup>1</sup>, A. Gomez<sup>1</sup>, L. Rodrigues Jacy da Silva<sup>1,2</sup>, C. Villaverde<sup>1,3</sup>, C. Velez<sup>1,3</sup>, A. Gimenez<sup>1,3</sup>, V. Lopez<sup>4</sup>, M. Ballesta<sup>4</sup>, J. Rosell<sup>5</sup>, E. Guillen<sup>6</sup>, M. J. Trujillo-Tiebas<sup>1,3</sup>, F. Blanco-Kelly<sup>1,3</sup>, C. Ayuso<sup>1,3</sup>, M. Corton<sup>1,3</sup>;

<sup>1</sup>Genetics Department, IIS-Universitary Hospital Fundación Jiménez Díaz, Madrid, Spain, <sup>2</sup>University of Mogi das Cruzes, São Paulo, Brazil, <sup>3</sup>Spanish Centre for Biomedical Network Research on Rare Diseases (CIBERER), ISCIII, Madrid, Spain, <sup>4</sup>Medical Genetics Department, University Hospital Virgen de la Arrixaca, Murcia, Spain, <sup>5</sup>Genetics Department, University Hospital Son Espases, Palma de Mallorca, Spain.

Introduction: Congenital eye malformations are highly heterogeneous conditions displaying a wide spectrum of overlapping phenotypes. Mutational load is not well known due to lack of systematic molecular characterization of large cohorts. Our aim is to perform comprehensive molecular screening for eye developmental causal genes using a next-generation sequencing (NGS) gene panel.

Methods: 82 uncharacterized patients with several ocular phenotypes (A/M, aniridia, ocular coloboma, anterior segment dysgenesis and nerve optic malformations) were analysed. Six positive cases carrying previously known mutations or CNVs were included for validation. A custom targeted enrichment system for 121 genes was used, followed by sequencing on Illumina platforms. Sanger validation and custom Agilent CGH arrays were used to validate candidate variants and CNVs, respectively.

Results: Currently we have findings for 44 patients. Targeted NGS identified pathogenic mutations or novel potentially damaging variants in 44% of patients. After segregation analysis and phenotyping, we characterized the 30% of cases, mainly those suffering from A/M. A total of 19 different causal mutations, being 63% of them novel, were found in 17 genes. Complete heterozygous deletion of OTX2 and SIX6 was also been identified by NGS in a patient with syndromic bilateral anophthalmia. Further aCGH analysis confirmed a 6.2Mb microdeletion at 14q22.3-q23.2 encompassing both genes.

Conclusions: Our custom NGS gene panel represents an accurate tool for genetic analysis of eye developmental diseases, allowing for a better understanding of pathogenic mechanisms, deciphering of novel phenotypic associations and improving molecular diagnosis of these rare conditions.

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## P02.25

### The importance of NGS in the diagnosis of syndromic deafness

*T. Likar<sup>1</sup>, N. Teran<sup>1</sup>, A. Maver<sup>1</sup>, M. Hasanhadžić<sup>2</sup>, B. Peterlin<sup>1</sup>, K. Writzl<sup>1</sup>;*  
<sup>1</sup>*Institute of Medical Genetics, Ljubljana, Slovenia, <sup>2</sup>UKC Tuzla, Tuzla, Bosnia and Herzegovina.*

**Objective:** 30% of patients with hereditary hearing loss have the syndromic form thereof. This means, that they have clinical signs of illness in at least one other organ system. Hearing loss has been described in more than 400 different syndromes but its severity varies widely. Our aim was to demonstrate the importance of NGS in the diagnosis of syndromic deafness when the patient's phenotype is atypical.

**Methods:** We enrolled 19 patients with HL who had previously been screened to exclude pathogenic mutations in the GJB2 gene and whose clinical symptoms indicated a form of syndromic deafness. Exon library construction and enrichment were performed using the Illumina TruSight One targeted panel and sequencing was subsequently carried out on the Illumina MiSeq Sequencer. Variant analysis and filtering were performed using a bioinformatic pipeline designed at our Institute.

**Results:** Our exome sequencing study led to the identification of 9 causative pathogenic variants among the 19 patients giving us a diagnostic yield of 47%. Variants were found in 8 different genes including CHD7, NEFL, MITF, HDAC8, SF3B4, USH2A, TSHZ1, and RYR1.

**Discussion:** Our results coincide well with previous findings that NGS has a high potential in the diagnosis of syndromic HL. We demonstrate that NGS is especially useful in the diagnosis of patients with atypical phenotypes and in cases of uncertainty of whether the HL is part of a syndromic case or a separate condition altogether.

## P02.26

### Noonan syndrome and related disorders associated with coloboma: seven case reports and review of literature

*Y. Capri<sup>1</sup>, H. Dridi<sup>1</sup>, F. Guimiot<sup>2,3</sup>, D. Héron<sup>4</sup>, M. Till<sup>5</sup>, N. Philip<sup>6</sup>, H. Dollfus<sup>7,8</sup>, E. Schaefer<sup>7</sup>, D. Timbolschi<sup>9</sup>, L. Vera<sup>9</sup>, P. Bitoun<sup>10</sup>, H. Cave<sup>11,12</sup>, A. Verloes<sup>1,3</sup>,*

<sup>1</sup>*Clinical genetics, CHU Robert Debré, Paris, France, <sup>2</sup>Biologie du développement, CHU Robert Debré, Paris, France, <sup>3</sup>INSERM UMR 1141, Paris, France, <sup>4</sup>Clinical genetics, CHU La Pitié Salpêtrière, Paris, France, <sup>5</sup>Cytogenetics, CHU Lyon, Bron, France, <sup>6</sup>Clinical genetics, CHU La Timone, Marseille, France, <sup>7</sup>Clinical genetics, CHU Strasbourg, Strasbourg, France, <sup>8</sup>CARGO, Strasbourg, France, <sup>9</sup>Pediatric ophthalmology, CHU Robert Debré, Paris, France, <sup>10</sup>Pediatric department, CHU Jean Verdier, Bondy, France, <sup>11</sup>Molecular genetics, CHU Robert Debré, Paris, France, <sup>12</sup>INSERM UMR S1131, Paris, France.*

Noonan syndrome (NS) is a multiple congenital malformation disease whose incidence is evaluated from 1/1000 to 1/2500 living births, quite frequent among the rare diseases. Characteristic features are short stature, facial dysmorphism, heart defects and cryptorchidism. Orthopedic, renal malformations, development delay and intellectual disability are also observed. Eyes complications are less described but very common since 95% of patients with NS displayed at least one or more ophthalmologic findings. Most of those findings are external features belonging to the characteristic facial dysmorphism of NS (hypertelorism, ptosis, down slanting palpebral fissures and epicanthic folds). Strabismus and refractive errors (myopia, hypermetropia and astigmatism) are also frequent. Fundus changes are reported less frequently. Few cases of iris and/or optic disc coloboma in patients with NS or related disorders have been described since 1987. Only one study focused on eye abnormalities in NS had found coloboma in 4% (2 cases among 51 patients). None of the previously NS cases with coloboma was confirmed by molecular analysis.

We describe 7 new cases of NS (or related disorders) with iris and/or optic disc coloboma and review the 10 previously reported cases. Elevated incidence of NS could let thought this association would be fortuitous but the 2 familial cases (one family previously reported and one new family) may suggest that coloboma is a rare complication of Rasopathy.

## P02.27

### Experimental pain threshold and tolerance analysis points to ROCK2 as a novel gene involved in nociception

*M. Kirin<sup>1</sup>, A. Miljković<sup>1</sup>, A. Relja<sup>1</sup>, A. Gelemanović<sup>1</sup>, C. Hayward<sup>2</sup>, M. Brašić<sup>3</sup>, I. Rudan<sup>4</sup>, I. Kolčić<sup>1</sup>, O. Polašek<sup>1</sup>;*

<sup>1</sup>*Medical School, University of Split, Split, Croatia, <sup>2</sup>Institute for Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>3</sup>Medical School, University of Zagreb, Zagreb, Croatia, <sup>4</sup>Usher Institute, University of Edinburgh, Edinburgh, United Kingdom.*

**Introduction:** The aim of this study was to investigate the genetic basis of acute pain.

**Materials and methods:** We measured experimentally-induced mechanical

pain threshold (least stimulus intensity) and pain tolerance (maximum tolerance stimulus intensity) in 1,114 previously genotyped subjects from the 10,001 Dalmatians cohort. The subjects were genotyped with either HumanHap 300v1 or 370CNV-Quad. QC protocol included removal of individuals with less than 97% genotyping rate, call rate below 98%, minor allele frequency below 2% and P-value for Fisher's exact test of Hardy-Weinberg equilibrium below 10E-10. Since subjects originated from three different groups, we performed a fixed effects meta-analysis.

**Results:** The results suggested one marginal hit for pain tolerance on chromosome 2, belonging to ROCK2 gene (rs4260216, P=5.3x10-7). The effect size of this SNP was comparable among all subsets, with similar allele frequencies and with very low heterogeneity (I<sup>2</sup>=0, P=0.540). This SNP also showed nominal significance association with pain threshold (P=6.8x10-4). There were no other SNPs in this region with suggestive significance, due to very low regional LD patterns.

**Conclusion:** This gene was previously implied in cytokinesis, smooth muscle contraction and focal adhesion, thus suggesting a plausible biological role in nociception, which was also recently implied in mice study. Replication efforts on humans should focus on independent population, larger sample sizes and possibly even patients with chronic pain.

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## P02.28

### Three major mutations and their rapid detection of the SLC26A4 gene in East Asian patients with Pendred syndrome/enlarged vestibular aqueduct syndrome

*T. KANAME<sup>1</sup>, K. Yanagi<sup>1</sup>, A. Ganaha<sup>2</sup>, M. Higa<sup>2</sup>, M. Iso<sup>1</sup>, Y. Matsui<sup>1</sup>, F. Koiwa<sup>1</sup>, Y. Kuroki<sup>1</sup>, M. Suzuki<sup>2</sup>, Y. Matsubara<sup>1</sup>;*

<sup>1</sup>*National Center for Child Health and Development, Tokyo, Japan, <sup>2</sup>University of the Ryukyus Graduate School of Medicine, Okinawa, Japan.*

Pendred syndrome (PS) and hearing loss associated with enlarged vestibular aqueduct (EVA) are caused by mutations in the SLC26A4 gene. We examined the SLC26A4 gene in 30 patients with PS or EVA in Okinawa Islands, whose clinical history, findings of physical and otoscopic examinations, hearing test, and computed tomography (CT) scan of the temporal bones were recorded, by Sanger sequencing and/or next-generation sequencing. We found that a mutation of IVS15+5G>A was the major allele in Okinawan patients and the 2nd was p.H723R, which was the major mutation allele in main Islands of Japan and in Korea. As ancestral differences have been reported between people from Okinawa Islands and those from the main islands of Japan, the mutation type of SLC26A4 was also different. According to our results and previous reports, the three mutations, IVS15+5G>A, p.H723R and IVS7-2A>G, accounted for more than 60% of SLC26A4 mutations in the East Asian patients. Thus, we constructed a simple and rapid detection system based on high resolution melting method for the three mutations. The detection system will be able to detect more than 95%, 60%, 90%, 65%, and 80% of SLC26A4 mutations in the Okinawan, Japanese, Korean, Chinese and Taiwanese patients, respectively. The system will be cost effective and will be applied to first screening/genetic testing of PS/EVA.

## P02.29

### Molecular study of 14 families with Perrault syndrome: genotype / phenotype correlations

*L. Jonard<sup>1</sup>, S. Marlin<sup>1</sup>, J. Lerat<sup>1</sup>, A. Munnich<sup>1</sup>, D. Lacombe<sup>2</sup>, S. Christin-Maitre<sup>3</sup>, C. Goizet<sup>2</sup>, L. Van Maldergem<sup>4</sup>, S. Gherbi<sup>4</sup>, P. Touraine<sup>5</sup>, J. Bonnefont<sup>6</sup>;*

<sup>1</sup>*Necker Hospital, APHP, Paris, France, <sup>2</sup>Bordeaux Hospital, Bordeaux, France, <sup>3</sup>Saint Antoine Hospital, APHP, Paris, France, <sup>4</sup>Besançon Hospital, Besançon, France, <sup>5</sup>Pitié Salpêtrière Hospital, APHP, Paris, France.*

Perrault syndrome is an autosomal recessive disorder characterized by the association of bilateral sensorineural hearing loss and female gonadal dysgenesis. The prevalence of this syndrome is probably underestimated due to the discovery of secondary ovarian failure and no sign associated with deafness in boys.

Recently five different genes: c100RF2, CLPP, HARS2, HSD17B4, and LARS2 were involved in Perrault syndrome. A sixth candidate gene has been suggested: LONP1.

Thanks to NGS technique, we analyzed the five known genes and the additional candidate gene in a cohort of 17 patients (14 families) with a clinical picture suggestive of Perrault syndrome, 6 patients presented with neurological signs. This is the first study of all the genes involved in Perrault syndrome in a cohort of patients.

We showed mutations in 8 families. LARS2 seems to be the most frequently

implicated gene and we haven't found any mutation in the candidate gene LONP1. 8 of the 10 mutations found in our study had not been described previously.

Thanks to our results and literature data (18 mutations, 31 patients, 12 families), we found correlations between the presence of neurological manifestations and mutations in HSD17B4 and c10ORF2 genes.

The absence of mutation in the studied genes in several families could be due either to a fortuitous association between ovarian dysgenesis and deafness in sporadic cases, or the existence of other genes involved. In favor of this hypothesis, a linkage study of a large family of patients allowed to exclude the six known loci.

#### P02.30

##### Genetics of Congenital Glaucoma in Saudi Arabia, phenotype-genotype correlation

*L. Abu Safieh, L. Aljasim, O. Owaydha, D. Edward;  
King Khaled Eye Specialist Hospital, riyadh, Saudi Arabia.*

Purpose: Primary congenital glaucoma (PCG) is an autosomal recessive disorder, affects children between birth and 3 years old and cases improper development of the eye's aqueous outflow system that results in optic nerve damage and significant vision loss. PCG is predominantly caused by mutations in the CYP1B1 gene. Aim was to understand more about the genetic factors verse the phonotypic presentation that affect the development and PCG in Saudi patients for proper diagnosis and patients care.

##### Methods

In this study we screened CYP1B1 in 75 PCG families at KKESH. DNA was extracted from affected individuals and available relatives. We performed PCR and sequence analysis to detect mutations and perform genotype/phenotype correlation.

Results: This study describes a missense mutation to be responsible for disease in less than 1% of the cases, the previously reported p.G61E mutation was found to be responsible for approximately 40% of all cases, emphasising on the founder effect expected from a consanguineous population like the Saudi population. Screening our cohort against the previously reported p.R469W mutation showed that it is responsible for approximately 1% of the cases. Approximately 34% were negative for CYP1B1 mutations, disease in these cases would be either due to regulatory or deep intronic mutations in CYP1B1 gene or other glaucoma known genes.

Conclusion: These results with the clinical evaluations are being used in genotype phenotype correlation and genetic counselling for family with PCG. We analysed age of onset, vision severity, myopia and other clinical features, significance was found between the 3 mutations group.

#### P02.31

##### Clinical and molecular characterization of the ABCA4-associated dystrophies: novel genotype-phenotype correlations

*M. Riera, A. Bures-Jelstrup, J. C. D'Antin, P. Mendez, B. Corcostegui, R. Navarro, E. Pomares;  
Institut de Microcirurgia Ocular, Barcelona, Spain.*

Purpose: Mutations in ABCA4 gene cause a wide range of recessive retinal disorders that can lead not only to macular dystrophies, but also more diffuse and peripheral atrophy patterns. To date, over 800 pathogenic variants have been associated to this gene, showing an extremely high allelic heterogeneity. The aim of this study was to evaluate whether certain ABCA4 alleles correlate with specific clinical features.

Methods: Clinical assessment was performed to characterize and classify a cohort of 55 families into five different phenotypes: Stargardt Disease (STGD), fundus flavimaculatus (FF), bull's eye maculopathy, cone dystrophy and cone-rod dystrophy (CRD). These families were genetically analyzed by a combination of highthroughput analyses, Sanger sequencing, genotyping microarrays, MLPA techniques, and cosegregation studies. When a new variant was detected, the pathogenic effect was validated using bioinformatic predictions, human polymorphism databases and molecular assays.

Results: In 62% of the families the two disease-causing alleles were found. Overall, 61 different ABCA4 variants were identified, including 18 novel ones. Our results showed a strong correlation between particular ABCA4-associated dystrophies and certain genotypes. Thus, the presence of two missense variants were mainly related with diffuse retinal disturbances such as FF and CRD, whereas STGD patients carried one missense mutation together with a null or splicing variant.

Conclusions: The clinical outcome of ABCA4-associated dystrophies may be determined by the type of the causative mutations. Our findings support that two missense variants are responsible for much more severe and diffuse phenotypes, suggesting that protein mislocalization or misfolding would cause more deleterious effects.

#### P02.32

##### Linkage analysis of Pakistani families with autosomal recessive retinal dystrophies

*Z. Ravesh<sup>1,2,3</sup>, B. Wissinger<sup>2</sup>, V. Yassaee<sup>1,4</sup>, M. Ansar<sup>3</sup>;*

*<sup>1</sup>Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Institute for Ophthalmic Research, Tübingen, Germany,*

*<sup>3</sup>Department of Biochemistry, Lab of Genomics, Quaid-i-Azam University, Islamabad, Pakistan, <sup>4</sup>Dept. of Medical Genetic, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of*

Introduction: Hereditary retinal dystrophies (RD) are a group of heterogeneous disorders caused by mutations in over 200 genes. RDs can be subdivided into different groups based on the primary degeneration of rod or cone photoreceptor cells. This study was conducted to investigate the underlying RD genes and mutation in consanguineous families from Pakistan.

Material and Methods: Families were recruited after informed consent. Peripheral blood was collected and genomic DNA was extracted according to standard procedures. Homozygosity mapping was performed using Affymetrix Gene Chip Human Mapping 250 K-NspI arrays. The data were analyzed using Homozygosity Mapper software. Primers for amplifying all exons and intron boundaries of all genes were designed with Primer3plus software followed by PCR and Sanger sequencing. Minigene splicing assay and DNA walking were performed on respective samples.

Results: Homozygosity mapping identified a novel locus in family A, one novel gene (C8ORF37) in family B and a large novel genomic deletion of the (LCA5) gene in family C.

Conclusion: Our study indicates the heterogeneous nature of retinal dystrophies in Pakistan. The amalgamation of traditional and modern molecular techniques is required for accurate identification of mutations. It is anticipated that these findings will contribute to future genetic testing in Pakistani families to minimize the risk of recessive disorders.

#### P02.33

##### Generation of retinal dystrophy mouse models by CRISPR/Cas9 genome editing

*M. López-Iniesta<sup>1,2,3</sup>, P. Escudero-Ferruz<sup>1</sup>, G. Marfany<sup>1,2,3</sup>, R. González-Duarte<sup>1,2,3</sup>,*

*<sup>1</sup>Dept. de Genética, Fac. Biología, Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, <sup>3</sup>CIBER-ER (Instituto Carlos III), Barcelona, Spain.*

Introduction: Mutations in over 200 genes are associated to inherited monogenic retinal degenerative diseases (prevalence 1:3000 worldwide), but we are still far from completely understanding their ethiopathology. Therefore, animal models are an essential tool to complement in vitro and cell culture assays. We aimed to generate two different mouse models by genome editing of CERKL and NR2E3, two retinal dystrophy genes, to dissect and characterize their precise role in photoreceptor cells.

Methods: We have generated mouse models by the new approach RNA-guided endonuclease CRISPR/Cas9 system. For CERKL, we performed a full gene deletion of nearly 100 kb. For NR2E3, we aimed to delete some of the functional domains. After zygote injections and embryo transfer, mosaic pups were genotyped to characterize the modified alleles, and were used as founder animals to obtain heterozygous homozygous mice in subsequent matings.

Results: As no viability problems were observed, experiments to assess the effect of CERKL and NR2E3 deletions in retinal phenotype are currently being carried out on wildtype, heterozygous and homozygous littermates. Retinal morphology and functionality is being assessed and compared to other knock-out and knock-down animal models. These new genetically modified strains will provide further insights into the role of these two genes in visual disorders.

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#### P02.34

##### Deubiquitinating enzyme genes as candidates for retinal disorders

*M. Esquerdo<sup>1,2</sup>, V. Abad-Morales<sup>1,2</sup>, V. Toulis<sup>1,2</sup>, G. Marfany<sup>1,2,3</sup>,*

*<sup>1</sup>Dept. de Genética, Fac. Biología, Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, <sup>3</sup>CIBER-ER (Instituto Carlos III), Barcelona, Spain.*

Introduction: Post-translational modifications, such as conjugation of ubiquitin, are crucial for the differentiation of specific retinal neurons and may play a role in photoreceptor cell fate. Ubiquitination is dynamic and reversible since the ubiquitin moiety is deconjugated from protein targets by deubiquitinating enzymes (DUBs). Many DUBs are implicated in human

pathological disorders from cancer and neurodegeneration to hereditary visual disorders. Besides, knockout or knockdown animal models of specific DUB genes show severe neuronal and eye phenotypes.

**Methods:** We aimed to characterize the gene expression pattern of several DUBs in the mouse retina. To this end, we detected mRNA and protein localization by *in situ* hybridization and fluorescent immunodetection. As a proof of principle, we also performed knockdown of selected DUB genes by morpholino microinjection in zebrafish embryos to study the resulting phenotype.

**Results:** Several expression patterns emerge, from ubiquitous expression to DUBs mostly detected in the photoreceptor layer or axonal processes, pointing to specific functions for different DUBs in the retina. Zebrafish knockdown morphants of selected DUBs show moderate to severe eye morphological defects, with defective formation of retinal structures: no lamination, no observable plexiform layers nor differentiated photoreceptors. These results support the relevance of some DUBs in the formation and differentiation of the vertebrate retina, making them good gene candidates for inherited retinal dystrophies or other visual disorders.

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#### P02.35

##### CNVs and mosaic are not an uncommon cause of retinal blindness in children

*I. Perrault<sup>1</sup>, S. Hanein<sup>2</sup>, N. Delphin<sup>3</sup>, J. Kaplan<sup>1</sup>, J. Rozet<sup>1</sup>*

<sup>1</sup>INSERM U1163, Lab of Genetics in Ophthalmology, IMAGINE Institut, Paris Descartes University, Paris, France, <sup>2</sup>INSERM U1163, NGS molecular diagnosis, IMAGINE Institut, Paris Descartes University, Paris, France, <sup>3</sup>Lab of Molecular diagnosis, Necker Hospital, Paris, France.

**Introduction.** Leber congenital amaurosis (LCA) and early-onset and severe retinal dystrophies (EOSRD) are a leading cause of incurable blindness in children. The visual outcome is variable, ranging from light perception to low but measurable visual acuity in the first two decades. LCA/EOSRD can be the presenting symptom in a range of devastating ciliopathies with skeletal, neurologic and/or renal involvement. Both the visual and extraocular outcomes strongly correlate with the gene. Here, we assessed targeted NGS (T-NGS) as a tool to improve the care of infants with severe visual deficiency. **Methods.** 300 index LCA/EOSRD cases were sequenced using a T-NGS array comprising 45 genes causing monosymptomatic and/or syndromic EOSRD and 10 genes of differential diagnoses. Mutations and familial segregation analysis were confirmed by Sanger sequencing.

**Results.** T-NGS allowed identifying disease-causing mutations in 184/300 (60 %) of the index cases: 10/184 had mutations that could not be detected by Sanger sequencing, including 9 heterozygote rearrangements (8 deletions, 1 duplication) and one mosaic (54 /395 reads), 6/184 had homozygous large deletions.

**Conclusion.** The molecular diagnosis in LCA/EOSRD neonates is crucial to predict the visual outcome, and to decide whether vital functions should be monitored. Yet, the genetic heterogeneity of these blinding conditions long challenged routine molecular testing. The identification of the mutation underlying the disease in 60% of the cases and the detection of second disease alleles missed by PCR-based Sanger sequencing in 5 % out of them support the relevance of implementing routine T-NGS for LCA/EOSRD.

#### P02.36

##### Functional role of CERKL, a Retinitis Pigmentosa gene, in mRNA transport in retinal cells

*R. Andres<sup>1,2,3</sup>, E. B. Domènech<sup>1,2,3</sup>, A. Pons-Pons<sup>4</sup>, M. J. López-Iniesta<sup>1,2,3</sup>, F. Sava<sup>1</sup>, G.*

*Marfan<sup>1,2,3</sup>, R. González-Duarte<sup>1,2,3</sup>*

<sup>1</sup>Barcelona University, Barcelona, Spain, <sup>2</sup>Institut de Biomedicina (IBUB), Barcelona University, Barcelona, Spain, <sup>3</sup>CIBERER, Instituto de Salud Carlos III, Barcelona, Spain

**Introduction:** CERKL mutations cause retinitis pigmentosa and cone-rod dystrophy in human. The CERKL gene is a paradigm for high transcriptional complexity, with many isoforms produced by alternative splicing and the use of alternative promoters. The protein displays several protein domains, such as lipid kinase, nuclear localization and nuclear export signals. Although the precise physiological function is still unknown, CERKL overexpression protects cells from apoptosis triggered by oxidative stress.

**Results:** In vitro studies on cultured cells showed that CERKL binds mRNA and contributes to stress granule complexes. These in vitro results have been further confirmed in isolated retinal neurons (retinal ganglion cells and photoreceptors), where CERKL co-localizes with RNA and RNA-binding proteins and is a component of the stress granules produced under oxidative stress conditions. Moreover, differential localization of CERKL isoforms in rods and cones has been shown using a panel of in-house antibodies.

**Conclusions:** This differential isoform specificity is highly suggestive of specific functional roles for CERKL in different photoreceptor cell types. Future work will address the impact of CERKL mutations in rods and cones related to human visual pathophysiology.

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#### P02.37

##### The molecular diagnosis of two large deletions in genes causing Retinitis Pigmentosa by WES

*M. de Castro-Miró<sup>1,2,3</sup>, R. Tonda<sup>4,5</sup>, B. Cormand<sup>1,2,3</sup>, R. González-Duarte<sup>1,2,3</sup>*

<sup>1</sup>Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain,

<sup>3</sup>CIBERER, Instituto de Salud Carlos III, Barcelona, Spain, <sup>4</sup>CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>5</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain.

Massive exome sequencing (WES) has revolutionized genetic diagnosis of highly heterogeneous monogenic disorders, as is the case of Retinitis pigmentosa (RP), the most frequent retinal disorder.

A heterozygous stop mutation was identified by WES in the *EYS* gene in an isolated RP patient. A subsequent dosage study of the *EYS* exons in the family was suggestive of a large deletion encompassing at least 10 exons, which was later supported by SNP cosegregation analysis. The identification of the second mutation confirmed *EYS* as the causative gene with autosomal recessive inheritance in the family. The second case was a large autosomal dominant RP pedigree with no plausible mutations revealed by WES in 5 affected members. Cosegregation of a rare *FGF21* variant with the disease in this family highlighted a closely linked RP gene (*CRX*) as a promising candidate. Coverage and SNP studies of the *CRX* genomic region in the patients, followed by genomic mapping with specific PCR primers, identified the boundaries of a heterozygous 10-kb deletion, spanning exons 3 and 4 of *CRX*. The benefits of WES for the molecular diagnosis of highly heterogeneous mendelian disorders are well established. However, the main drawback is the identification of gross structural genomic alterations at heterozygosity by using standard variant calling strategies. In such cases, the genetic data can be decisive to attain the genetic diagnosis by pointing to the disease candidates.

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#### P02.38

##### Towards rapid genetic diagnosis of developmental eye anomalies: design and implementation of a large diagnostic and research gene panel

*J. L. Brutly<sup>1</sup>, Y. Wallis<sup>1</sup>, D. McMullan<sup>1</sup>, S. Clokie<sup>1</sup>, M. Griffiths<sup>1</sup>, R. Holt<sup>2</sup>, D. Bax<sup>2</sup>, J. Hoffman<sup>3</sup>, S. Hadfield<sup>3</sup>, N. Ragge<sup>2,3</sup>*

<sup>1</sup>West Midlands Regional Genetics Laboratory, Birmingham, United Kingdom, <sup>2</sup>Oxford Brookes University, Oxford, United Kingdom, <sup>3</sup>Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, United Kingdom.

Developmental eye anomalies, including anophthalmia, microphthalmia and coloboma, affect ~1 in 3,500 individuals worldwide. At least 150-200 genes are implicated in these conditions, with many other genes known to be important in eye development pathways, and still more to be identified. Currently 4-5 genes are tested routinely in the UK, meaning only 28% of developmental eye anomalies patients receive genetic diagnosis; often following a lengthy research input.

A diagnostic gene panel containing 351 genes known to be involved in eye development and 195 genes of potential interest was developed, using the Illumina Nextera Rapid Capture Custom Enrichment kit, and sequenced using the MiSeq platform. Sequence data was processed using an in-house bioinformatics pipeline. A validation cohort of 10 patients who have previously undergone research testing, along with 16 new patients have been tested so far, with a further 16 patients planned.

So far mutations have been identified in 15 genes including SOX2, PAX6, FOXE3 and BEST1, including all known mutations from the validation cohort and definite causative pathogenic mutations in 2/16 new patients (12%). Several other gene alterations are undergoing further analysis. A large amount of research data has also been collected, and is linked to an adjunctive research programme for further analysis.

A large panel for sequencing mutations in patients with developmental eye

anomalies is a valid and viable approach, and helps reduce the time taken for diagnosis. Including research genes linked to a research programme also gives huge potential benefit for further understanding of developmental eye anomalies.

#### P02.39

#### Splicing defects caused by rare *OPN1LW/MW* haplotypes associated with X-linked cone dysfunction disorders: *de novo* intrachromosomal gene conversion from *MW* to *LW* in the male germline results in Blue Cone Monochromacy

E. Buena Atienza<sup>1</sup>, K. Ruether<sup>2,3</sup>, B. Baumann<sup>1</sup>, R. Bergholz<sup>2</sup>, N. Weisschuh<sup>1</sup>, S. Kohl<sup>1</sup>, B. Wissinger<sup>1</sup>;

<sup>1</sup>Institute of Ophthalmic Research, Tübingen, Germany, <sup>2</sup>Charité Campus Virchow-Klinikum, Berlin, Germany, <sup>3</sup>Sankt Gertrauden-Krankenhaus, Berlin, Germany.

X-linked cone dysfunction disorders such as Blue Cone Monochromacy and X-linked Cone Dystrophy are characterized by absent or reduced L- and M- cone function due to defects in the *OPN1LW/OPN1MW* gene cluster. We studied 24 affected males from 16 families with either a structurally intact gene cluster or at least one intact single (hybrid) gene, but carrying rare combinations of common SNPs in exon 3 in single or multiple *OPN1LW* and *OPN1MW* gene copies. We applied a semi-quantitative minigene splicing assay to assess twelve different *OPN1LW/MW* exon 3 haplotypes. Nine haplotypes resulted in aberrant splicing of equal or more than 20% of transcripts including the known pathogenic haplotypes (i.e. 'LIAVA', 'LVAVA') with absent or minute amounts of correctly spliced transcripts, respectively. In one family with strikingly different phenotypes we observed *de novo* occurrence of the 'LIAVA' haplotype derived from an ancestral less deleterious 'LIAVS' haplotype. We could establish an intrachromosomal gene conversion in the male germline as the underlying mechanism driving the phenotype change from macula dystrophy with deuteranopia into Blue Cone Monochromacy. The converted sequence was narrowed to a region of ~1300 bp originating from a downstream *OPN1MW* gene. Although gene conversion shaping the *OPN1LW/OPN1MW* genes has been postulated and inferred from new arising mutations, we are the first to demonstrate a *de novo* gene conversion within the lineage of a pedigree.

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#### P02.40

#### Identification and functional assessment of deep-intronic and non-canonical splice defects in *ABCA4* associated with Stargardt disease

R. Sangermano<sup>1,2</sup>, S. Albert<sup>1,2</sup>, M. Bauwens<sup>3</sup>, N. Bax<sup>4</sup>, S. Cornelis<sup>1</sup>, V. Richelle<sup>1</sup>, L. I. van den Born<sup>5</sup>, A. Garanto<sup>1,2</sup>, C. B. Hoyng<sup>4</sup>, R. W. J. Collin<sup>1,2</sup>, E. De Baere<sup>3</sup>, F. P. M. Creemers<sup>1,2</sup>;

<sup>1</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>Radboud Institute of Molecular Life Sciences, Nijmegen, Netherlands,

<sup>3</sup>Center for Medical Genetics, Ghent University and Ghent University Hospital, Ghent, Belgium, <sup>4</sup>Department of Ophthalmology, Radboud University Medical Center, Nijmegen, Netherlands, <sup>5</sup>Rotterdam Eye Hospital, Rotterdam, Netherlands.

**Purpose:** Non-canonical *ABCA4* splice variants are frequent in Stargardt disease (STGD1), but their effect is poorly understood. Deep-intronic variants have also been shown to affect RNA splicing. About 30% of STGD1 patients carry one or no *ABCA4* variant, and we hypothesize that the missing mutations reside in the non-coding parts. Transcript analysis is not straightforward as *ABCA4* is specifically expressed in the retina. The purpose of this study is to (1) identify intronic variants in unsolved patients by *ABCA4* locus sequencing, and (2) to prove their functional effects as well the effect of non-canonical splice variants, by *in vitro* splice assays.

**Methods:** Haloplex-based locus sequencing was performed in 70 mono-allelic maculopathy cases. After applying splice site prediction programs, the effect of intronic variants was tested *in vitro* by cloning ~1.5-kb fragments into minigenes, transfecting them into HEK393T cells and performing reverse transcription-PCR.

**Results:** Haloplex sequencing revealed > 80 rare deep-intronic variants which potentially activate cryptic exons. At least 12 are being analyzed by minigene splice assays. The non-canonical c.5461-10T>C and c.6729+5\_6729+19del variants were shown to result in exon 39/40 or 48 skipping, respectively, effectively resulting in null alleles.

**Conclusions:** Two non-canonical splice variants, among which the most frequent severe *ABCA4* variant, c.5461-10T>C, were shown to completely inactivate *ABCA4* function. Minigene splice assays are effective to investigate variants predicted to have an effect on splicing. Some of these variants might be amenable for antisense oligonucleotide-based therapy.

#### P02.41

#### Molecular genetic analysis of *COL2A1* gene in a group of Czech patients with Stickler syndrome

J. Sabova<sup>1,2</sup>, V. Suchá<sup>1</sup>, M. Simandlova<sup>2</sup>, V. Horinova<sup>3</sup>, R. Pourova<sup>2</sup>, A. Krepelova<sup>2</sup>;

<sup>1</sup>Laboratory for Molecular Diagnostics, Synlab Genetics s.r.o., Prague, Czech Republic,

<sup>2</sup>Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, <sup>3</sup>Sanatorium Helios, Brno, Czech Republic.

Stickler syndrome (STL) is an AD progressive disorder of connective tissue and it is one of the most frequent genetic syndromes associated with deaf-blindness with incidence 1:7500-1:9000 newborns. STL is characterized by midfacial underdevelopment and cleft palate (either alone or as part of the Robin sequence); eye abnormalities; hearing loss (both conductive and/or sensorineural); and mild spondyloepiphyseal dysplasia and/or precocious arthritis. Eye findings include high myopia, cataract, retinal detachment leading to impaired vision or blindness. The degree of hearing loss varies among affected individuals and may become more severe over time. There are several types of STL, which are distinguished by their genetic cause and their characteristic signs and symptoms. The most common type I (STL1) is responsible for approximately 70% of reported cases and presents with a wide variety of symptoms affecting the eye, ear, facial appearance, palate and musculoskeletal system and occurs due to mutations over the entire *COL2A1* gene on chromosome location 12q13.11. In our pilot study we investigated 29 patients from 15 families with STL from the Czech Republic (CR). We analysed the *COL2A1* gene using Sanger sequencing as a prescreening and here we present the first 3 families in the Czech Republic with 2 detected known heterozygous frameshift mutations c.2382delT and one novel splice-site mutation c.1123-2A>G. Identification of these mutations confirmed the diagnosis of Stickler syndrome. NGS screening of *COL2A1* negative patients will follow.

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#### P02.42

#### TMPRSS3 mutation as a cause of non-syndromic hearing impairment among Polish hearing loss patients

U. Lechowicz<sup>1</sup>, A. Pollak<sup>1</sup>, A. Podgorska<sup>1</sup>, P. Stawinski<sup>1</sup>, M. Oldak<sup>1</sup>, H. Skarzynski<sup>2</sup>, R. Płoski<sup>3</sup>;

<sup>1</sup>Institute of Physiology and Pathology of Hearing, Department of Genetics, Warsaw, Poland, <sup>2</sup>Institute of Physiology and Pathology of Hearing, Warsaw, Poland, <sup>3</sup>Medical University of Warsaw, Department of Medical Genetics, Centre of Biostructure, Warsaw, Poland.

Recessive mutations of the TMPRSS3 gene cause non-syndromic hearing impairment (HI) but little is known about their spectrum among Polish population. The purpose of the study was to search for pathogenic TMPRSS3 variants in Polish HI patients, determine the pathogenicity of those variants and genotype-phenotype correlations.

Using WES (Illumina HiSeq1500) in one patient two mutations in the TMPRSS3 gene were found. Previously, mutations in the GJB2 gene, common deletions in the GJB6 gene and mitochondrial m.3243A>T and m.1555A>T mutations were excluded in this patient. A large cohort of HI patients (n=2277) and a control group (n=500) were studied to assess the prevalence of TMPRSS3 mutations with real-time PCR. Sanger sequencing was used to confirm the presence of the detected TMPRSS3 variants and to search for the second mutation in the TMPRSS3 gene.

We found 43 (1.88%) probands with 14 different TMPRSS3 variants. The most frequent mutation was the already known variant p.H70Tfs\*19, the second one was p.A138E. Truncating TMPRSS3 mutations cause a more severe HI with an earlier onset than missense mutations. The p.A90T variant previously linked with HI had a high prevalence in controls (~6%) indicating that is non-pathogenic change.

Mutations in TMPRSS3 gene are not a common cause of HI in patients with autosomal recessive hearing loss, but considering the group of patients with a partial deafness HI and postlingual age of onset, TMPRSS3 gene mutations may be an important component.

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## P02.43

### Pathogenic Role of the USH2A mutation (p.Cys759Phe) among Spanish families with Retinitis Pigmentosa or Usher syndrome

R. Perez-Carre<sup>1</sup>, A. Avila-Fernandez<sup>2</sup>, F. Blanco-Kelly<sup>1</sup>, A. Gimenez-Pardo<sup>1</sup>, I. Martin-Merida<sup>1</sup>, M. Corton<sup>1</sup>, N. Reyes<sup>1</sup>, E. Aller<sup>2</sup>, J. Millan<sup>2</sup>, C. Ayuso<sup>1</sup>;

<sup>1</sup>Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Madrid, Spain, <sup>2</sup>Instituto de Investigación Sanitaria IIS-La Fe, Valencia, Spain.

Mutations in USH2A have been associated with non-Syndromic Retinitis Pigmentosa (RP) and Usher syndrome (USH), being the p.Cys759Phe one of the most frequent mutation. A recent study (Gonzalez-del Pozo et al) has questioned the pathogenicity of this variant detected homozygously in two asymptomatic individuals. However, the high prevalence of the p.Cys759Phe mutation in our cohort of patients supports its pathogenicity. The aim of this study was to establish the implication of p.Cys759Phe mutation as a cause of syndromic or non-syndromic RP.

A total of 81 Spanish families affected with RP or USH, carrying the p.Cys759Phe mutation, either in homozygosity or heterozygosity, were analyzed using classical molecular techniques. Among them, 15 heterozygous cases were further studied by different targeted-NGS approaches in order to identify the second causative allele.

We could characterize 68 families being 12 of them homozygous for the p.Cys759Phe variant. Furthermore, fifty-three compound heterozygous for USH2A were also found. In three out of 68 characterized patients, causative mutations in a different gene were identified being also carriers of the p.Cys759Phe. The remaining 13 uncharacterized families with the p.Cys759Phe in heterozygosity are being analysed by NGS for detecting disease-causing mutation.

All these data together seem to point to the pathogenic causative role for p.Cys759Phe, although another modifier effect for RP cannot be excluded. Overall, a comprehensive study of the molecular basis of these diseases allows us to provide an accurate diagnosis, a prognosis of the disease and proper genetic counseling to the patients.

F J D - B i o b a n c o ( R D 0 9 / 0 0 7 6 / 0 0 1 0 1 ) , C I B E R - ER(06/07/0036), FIS(PI013/00226 and PI013/00638), Fundación Conchita Rábago, CP/03256 (ISCIII).

## P03 Internal organs & endocrinology (lung, kidney, liver, gastrointestinal)

### P03.01

#### Different ethnical distribution of the aberrant splice mutation in intron 2, c. 293-13A/C>G, at CYP21A2 gene in Macedonian patients with classical form of 21-hydroxylase deficiency

V. Anastasovska, M. Kocova;

Genetic Laboratory, Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Macedonia, The Former Yugoslav Republic of.

Introduction: 21-hydroxylase deficiency is an autosomal recessive endocrine disorder due to mutations in the CYP21A2 gene. Severe enzyme deficiency can present as a classical salt wasting (SW) and simple virilizing form (SV). The In2G (c.293-13A/C>G) mutation provides ~ 2% enzyme activity. It encompasses 25% of the classic 21-hydroxylase deficiency alleles and 51% of the SW alleles.

Materials and Methods: We have performed molecular analysis of the In2G (c.293-13A/C>G) mutation in 49 Macedonian patients with clinical and laboratory signs of severe form of 21-hydroxylase deficiency, 26 SW and 23 SV form, evaluated at the Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Republic of Macedonia, using differential PCR/ACRS. Of the patients, 27 had Macedonian, 14 Albanian, and 8 Roma ethnic origin.

Results: The In2G mutation was detected in 57.1% of the patients, 40.8% were homozygotes and 16.3% were heterozygotes. It was revealed in 65.4% (34/52) of the SW alleles, and 30.4% (14/46) of the SV alleles. Interestingly, In2G was detected in all Roma patients on 93.8% of the alleles (7 homozygotes and 1 heterozygote), followed by 57.1% of the Albanians on 53.6% alleles (7 homozygotes and 1 heterozygote), and 44.4% of the Macedonian patients on 33.3% of the alleles (6 homozygotes and 6 heterozygote).

Conclusions: We found different ethnic distribution of the In2G mutation in the Macedonian patients with severe 21-hydroxylase deficiency. High In2G frequency detected in our patients, that is comparable to the most of the other European countries, supports its role in classical phenotype of the 21-hydroxylase deficiency.

### P03.02

#### Dysregulation of NR5A1 is a novel and recurrent cause of 46,XX (ovo) testicular disorder of sex development

D. Baetens<sup>1</sup>, A. L. Todeschini<sup>2</sup>, F. Peelman<sup>3</sup>, H. Stoop<sup>4</sup>, F. Coppieters<sup>1</sup>, T. Rosseel<sup>1</sup>, T. Van Laethem<sup>1</sup>, L. H. Looijenga<sup>4</sup>, R. A. Veitia<sup>2</sup>, M. Cools<sup>5</sup>, E. De Baere<sup>1</sup>;

<sup>1</sup>Center for Medical Genetics, Ghent University, Ghent, Belgium, <sup>2</sup>Institut Jacques Monod, Université Paris Diderot, Paris, France, <sup>3</sup>Department of Medical Protein Research, Faculty of Medicine and Health Sciences, Flanders Institute for Biotechnology, Ghent, Belgium, <sup>4</sup>Department of Pathology, Erasmus MC-University Medical Center Rotterdam, Rotterdam, Netherlands, <sup>5</sup>Department of Pediatrics, Division of Pediatric Endocrinology, Ghent University Hospital and Ghent University, Ghent, Belgium.

Background: While many causes of 46,XY disorders of sex development (DSD) have been elucidated, the mechanisms leading to 46,XX (ovo)testicular DSD are poorly understood. Both conditions may represent a phenotypic spectrum resulting from a common underlying genetic defect.

Methods: Whole exome sequencing was applied to identify the molecular cause in ten unrelated patients with 46,XX (ovo)testicular DSD. Transcriptional activation of the variant of interest was assessed using luciferase assays. RNA-seq was performed on patient-derived lymphocytes. Immunohistochemistry was done on gonadal specimens for markers of gonadal development.

Results: We identified a novel heterozygous NR5A1 variant c.274C>T p.(Arg92Trp) in three unrelated cases with 46,XX (ovo)testicular DSD. This variant is absent in genomic databases and is predicted to be deleterious. The Arg92 residue is conserved up to zebrafish and located in the RGGR motif before the C-terminus helix in the conserved Ftz-F1 box, likely affecting DNA-binding stability and specificity. Immunohistochemistry confirmed SRY-independent SOX9 expression and absence of FOXL2 in testicular parts of XX gonads and vice versa in ovarian regions. No consistent changes in transcriptional activation were seen. RNA-seq showed upregulation of MAMLD1, a direct target of NR5A1.

Conclusions: Previously, loss-of-function mutations in NR5A1 were found in a spectrum of male undervirilization. Here, a novel NR5A1 mutation was found in three out of ten cases with 46,XX (ovo)testicular DSD, an ultra-rare condition. We hypothesize that this variant results in gain-of-function and triggers testicular differentiation in 46,XX individuals. We propose NR5A1 mutation p.(Arg92Trp) as a novel and recurrent cause of 46,XX (ovo)testicular DSD.

### P03.03

#### Dissecting the genetic complexity of Addison's disease by using targeted resequencing data of a large Swedish cohort

M. Bianchi<sup>1</sup>, D. Eriksson<sup>2</sup>, J. Wahlberg<sup>3</sup>, O. Ekwall<sup>4</sup>, P. Dahlqvist<sup>5</sup>, A. Hulting<sup>6</sup>, K. Lindblad-Toh<sup>1,7</sup>, S. Bensing<sup>8</sup>, O. Kämpe<sup>2</sup>, G. Rosengren Pielberg<sup>9</sup>;

<sup>1</sup>Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, <sup>2</sup>Center for Molecular Medicine, Department of Medicine, Karolinska Institutet, Stockholm, Sweden, <sup>3</sup>Department of Endocrinology, and Department of Medical and Health Sciences, and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, <sup>4</sup>The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, <sup>5</sup>Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden, <sup>6</sup>Department of Molecular Medicine and Surgery, Karolinska Institutet, and Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden, <sup>7</sup>Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, MA, United States.

Addison's disease (AD) is a rare autoimmune disorder characterized by the failure of the adrenal cortex. Besides HLA and a few shared autoimmunity-predisposing associated loci, the genetics underlying AD is largely unknown. The Swedish Addison registry consists of 700 AD patients accurately characterized regarding their clinical phenotype and autoimmune serological parameters. 83% of the patients have 21-hydroxylase autoantibodies and 62% have at least another autoimmune disease. We resequenced 1,900 target genes involved in immune function and autoimmunity in all AD patients and in 1,500 healthy controls. Targets included coding sequences, full UTRs and conserved intronic and intergenic regions. GATK, LASER and KING software were used to call variants, pinpoint predicted non-Europeans and identify related samples, respectively. Single SNP and aggregate association tests, as well as pathway analysis will be performed in order to confirm known and unravel novel loci associated with AD and its co-inherited autoimmune diseases in the Swedish population.

We expect that our study of a slightly unconstrained Swedish population isolate will enable to improve global diagnostic and medical AD treatments by finding disease variants likely to be causative across different populations.

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## P03.04

### Exome sequencing identifies a novel recessive subtype of colorectal adenomatous polyposis

R. Adam<sup>1</sup>, I. Spier<sup>1</sup>, B. Zhao<sup>2</sup>, M. Kloth<sup>3</sup>, I. Hinrichsen<sup>4</sup>, N. Friedrichs<sup>3</sup>, J. Marquez<sup>2</sup>, S. Horpaapan<sup>5</sup>, J. Kirfel<sup>6</sup>, J. Altmüller<sup>7,8</sup>, A. Laner<sup>9,10</sup>, S. Holzapfel<sup>11</sup>, S. Peters<sup>1</sup>, K. Kayser<sup>1</sup>, H. Thiele<sup>9</sup>, G. Kristiansen<sup>9</sup>, E. Holinski-Feder<sup>10,11</sup>, M. M. Noethen<sup>1,12</sup>, R. Buettner<sup>1</sup>, A. Brieger<sup>4</sup>, R. P. Lifton<sup>13</sup>, S. Aretz<sup>1</sup>;

<sup>1</sup>Institute of Human Genetics, Bonn, Germany, <sup>2</sup>Department of Genetics, New Haven, CT, United States, <sup>3</sup>Institute of Pathology, Cologne, Germany, <sup>4</sup>Medical Clinic 1, Biomedical Research Laboratory, Frankfurt, Germany, <sup>5</sup>Department of Anatomy, Faculty of Medical Science, Phitsanulok, Thailand, <sup>6</sup>Institute of Pathology, Bonn, Germany, <sup>7</sup>Institute of Human Genetics, Cologne, Germany, <sup>8</sup>Cologne Center for Genomics, Cologne, Germany, <sup>9</sup>Ludwig-Maximilians-University Munich, Munich, Germany, <sup>10</sup>Medical Genetics Center, Munich, Germany, <sup>11</sup>Ludwig-Maximilians-University, Munich, Germany, <sup>12</sup>Department of Genomics, Life & Brain Center, Bonn, Germany, <sup>13</sup>Institute of Genetics, New Haven, CT, United States.

**Background:** In around 30% of families with colorectal adenomatous polyposis, no germline mutation in the known genes APC, MUTYH, POLE, POLD1, or NTHL1 can be identified, although a hereditary etiology is likely. **Methods:** To uncover new high-penetrance causative genes, exome sequencing of leukocyte DNA from 104 unrelated patients with unexplained adenomatous polyposis was performed. For data analysis and variant filtering, an established bioinformatics pipeline including in-house tools was applied. **Results:** We identified two unrelated patients with differing compound-heterozygous loss-of-function germline mutations in a DNA repair gene. The pedigrees, genotypes, and the frequency of mutations of this gene in the general population are consistent with an autosomal recessive mode of inheritance. By transcriptional analysis, by functional analysis of the tumor tissue and by use of various in-silico tools we found strong evidence for a loss-of-function effect. No germline mutation in any other gene known to cause polyposis was identified in these patients. Both index patients had an affected sibling carrying the same mutations. Besides colorectal and duodenal adenomas, colorectal cancer, gastric cancer, and early onset astrocytoma were reported in the two patients and their affected siblings. In addition, we identified one unrelated patient with a biallelic PMS2 germline mutation, representing Constitutional Mismatch Repair Deficiency Syndrome (CMMRD). **Conclusions:** The present study is the first to identify biallelic pathogenic germline mutations of the given DNA repair gene in patients with a suspected hereditary tumor syndrome. Our data suggest that the reported cases represent a novel recessive subtype of colorectal adenomatous polyposis.

## P03.05

### Molecular analysis of AR gene in fourteen female patients with 46,XY - advances of two generations of sequencing

S. Andonova<sup>1</sup>, R. Vazharova<sup>2,3</sup>, L. Grozdanova<sup>4</sup>, R. Robeva<sup>5</sup>, E. Stefanova<sup>6</sup>, L. Balabanski<sup>3</sup>, S. Ivanov<sup>3</sup>, D. Toncheva<sup>7</sup>, P. Kumanov<sup>5</sup>, M. Sirakov<sup>6</sup>, A. Savov<sup>1</sup>;

<sup>1</sup>National Genetic Laboratory, UHOG „Maichin dom“, Sofia, Bulgaria, <sup>2</sup>Sofia University „St. Kliment Ohridski“, Sofia, Bulgaria, <sup>3</sup>GARH Malinov, Genomic Laboratory, Sofia, Bulgaria,

<sup>4</sup>Department of Pediatrics and Medical Genetics, Medical University Plovdiv, Plovdiv, Bulgaria, <sup>5</sup>Clinical Center of Endocrinology and Gerontology, Medical University – Sofia, Sofia, Bulgaria, <sup>6</sup>Pediatric Endocrinology, Diabetes and Genetics Clinic, University Pediatric Hospital, Medical University-Sofia, Sofia, Bulgaria, <sup>7</sup>Department of Medical Genetics, Medical University - Sofia, Sofia, Bulgaria, <sup>8</sup>Department of Gynecological endocrinology and Pediatric and Adolescent Gynecology, UHOG „Maichin dom“, Medical University – Sofia, Sofia, Bulgaria.

Disorders of sex development (DSD) are group of heterogeneous conditions that usually present with atypical genitalia and/or delayed puberty. One of the most common disorders in this group is the Androgen Insensitivity Syndrome (AIS) - the affected individuals are phenotypically females with resistance to androgen action and 46,XY karyotype. More than 600 mutations associated with the variable phenotype of the disorder are known in Androgen Receptor (AR) gene (Xq12). Fourteen XY female patients were referred to our team due to primary amenorrhea and/or discordance between phenotypic and chromosomal sex. Molecular analysis of AR gene was conducted using Sanger sequencing (9 patients) and targeted Next-generation sequencing (5 patients). Mutations in 6 patients were found: p.Q147Ter, p.R616H, p.R780W and p.I842N. The mutation was inherited from the heterozygous mother in 4 of the cases. The mother of the one patient was not a carrier and the mutation was de novo. DNA sample from relatives of the last patient were not available. Further analyses revealed mutations in SRY, SRD5A2 and NR5A1 genes in three of the remaining eight cases.

**Conclusion:** Sanger sequencing of AR is indicated for patients with strong suspicions for AIS while use of targeted next-generation sequencing of known genes associated with DSD could be beneficial in cases with atypical

phenotype and clinical data. The combination of routine and new genetic technologies could be crucial for the molecular diagnosis in such genetically heterogenous diseases as 46,XY DSD.

## P03.06

### Identification by exome sequencing and functional characterization of nonobvious pathogenic variants responsible for Alport syndrome

C. Chiereghin<sup>1,2</sup>, M. Robusto<sup>1,2</sup>, P. Castorina<sup>3</sup>, M. Gian<sup>3</sup>, S. Duga<sup>1,2</sup>, R. Asselta<sup>1,2</sup>, G. Solda<sup>4,2</sup>,

<sup>1</sup>Humanitas University, Rozzano (Milan), Italy, <sup>2</sup>Humanitas Clinical and Research Center, Rozzano (Milan), Italy, <sup>3</sup>Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy.

**Introduction:** Alport syndrome (AS) is a progressive inherited renal disease caused by mutations in three large collagen-IV genes (COL4A3, COL4A4, COL4A5), comprising about 50 exons each. Recently, the introduction of next-generation sequencing allowed the analysis of all three AS genes in a single step. Despite that, the total mutation detection rate has been estimated between 55-80%.

**Materials and Methods:** We selected three Italian families with clear clinical evidence of AS but no molecular diagnosis, despite having been subjected to extensive candidate gene analyses. The probands were screened by whole-exome sequencing (WES) using the Nextera Rapid Capture kit on a Next-Seq500 platform. All exons of AS genes were adequately covered.

**Results:** Analysis of variants within AS genes identified pathogenic mutations in all families. In Family-1, we identified an homozygous 24-bp in-frame deletion in COL4A3 (p.Val11\_Leu18del), segregating with autosomal recessive AS. The variant is predicted to disrupt the signal peptide, possibly altering COL4A3 protein secretion. In Family-2 proband, a COL4A5 hemizygous missense mutation (p.Gly491Asp) was found in exon 33, which was not covered by previous targeted resequencing. In Family-3, we detected an heterozygous intronic variant (c.2245-40A>G) altering COL4A5 exon-29 branch site, as we confirmed by in-vitro studies. Analysis of the pattern of X-chromosome inactivation (XCI) on DNA from blood revealed a correlation between unbalanced XCI and phenotypic manifestations in mutant females. **Conclusions:** WES was fundamental to provide a molecular diagnosis in 3 AS families, highlighting non-obvious pathogenic variants that escaped previous screenings.

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## P03.08

### The role of DNA analysis in diagnosis of autosomal recessive polycystic kidney disease

L. Obeidova<sup>1</sup>, V. Elisakova<sup>1</sup>, T. Seeman<sup>2</sup>, J. Reiterova<sup>3</sup>, J. Stekrova<sup>1</sup>;

<sup>1</sup>Institute of Biology and Medical Genetics of the First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic,

<sup>2</sup>Department of Paediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital in Prague, Prague, Czech Republic, <sup>3</sup>Department of Nephrology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic.

**Introduction:** Autosomal recessive polycystic kidney disease (ARPKD) is a severe form of chronic kidney disease, frequently diagnosed prenatally. ARPKD is caused by mutations in the PKHD1 gene, nevertheless, the same phenotype is part of many other disorders. Thus, the molecular genetic analysis can be very useful in differential diagnosis of ARPKD.

Herein, we present the results of molecular genetic analysis in 52 families with clinically suspected ARPKD.

**Materials and methods:** The analysis of PKHD1 was carried out using next-generation sequencing and subsequent MLPA (multiplex ligation-dependent probe amplification). In some of the families, genetic analysis of HNF1 $\beta$ , PKD1/2 was carried out. The cohort of probands was divided into 2 groups (Group A and B) on the basis of their fulfillment of clinical criteria of ARPKD. Patients fulfilling all criteria were placed in Group A (n=24). Group B consisted of 30 patients.

**Results:** The detection rate in PKHD1 amounted to 81% in Group A, and 27% in Group B. Six families within Group B without mutation in PKHD1 harbored mutations in other genes (HNF1 $\beta$ , PKD1/2 and NPHP11).

**Conclusions:** The detection rate of PKHD1 mutations in children fulfilling all clinically diagnostic criteria of ARPKD is high, reaching 81%. However, mutations of PKHD1 were also detected in some of the patients without having met all three clinical criteria, especially in patients who died perinatally. The important sign of ARPKD in children proved to be congenital hepatic fibrosis.

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## P03.09

### Introduction of a routine NGS-based genetic screening into the universal screening programme for hypercholesterolemia in Slovenian 5-years old children

**J. Kovač**, G. Klančar<sup>1</sup>, T. Tesovnik<sup>1</sup>, U. Grošelj<sup>2</sup>, T. Battelino<sup>2,3</sup>, K. Trebušak Podkrajšek<sup>1,3</sup>,  
<sup>1</sup>Unit of Special Laboratory Diagnostics, University Children's Hospital, UMC, Ljubljana, Slovenia, <sup>2</sup>Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC, Ljubljana, Slovenia, <sup>3</sup>Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

#### Introduction:

Untreated familial hypercholesterolemia (FH) increases the risk for developing atherosclerosis and cardio-vascular disease in early adulthood for up to 100-fold. Slovenia launched the program of universal screening for hypercholesterolemia in 5-year-old children in 1995 with routine measurement of fasting serum cholesterol. Additionally, the routine genetic screening using NGS technology was introduced in 2011 to identify causative genetic variants in individuals with positive result of the screening.

#### Materials and Methods:

272 individuals (149 females) were sent to a tertiary paediatric outpatient clinic due to the increased level of serum cholesterol and referred to genetic testing. Genomic DNA was isolated from whole-blood samples according to established laboratory protocol. Samples for NGS were prepared using ADH MASTR assay (Multiplicom, Belgium) for detection of variants in 4 genes associated with FH (LDLR, PCSK9, APOE and exon 26 of APOB) and sequenced on a MiSeq platform with MiSeq reagent kit v2 (Illumina, California) following the manufacturer's protocol.

#### Results:

105 (38.6%) of individuals had causative variant identified in LDLR gene followed by 55 individuals (18.4%) with causative variant in APOB and none in PCSK9. Additionally, 51 participants (18.7%) were carriers of APOE E4 isoform genotype. The results of genetic analysis were negative for 66 participants (24.3%).

#### Conclusions:

Results clearly demonstrated the efficacy of universal screening programme with most participants having genetically confirmed FH. Additionally, data for family history may not suffice for reliable identification of FH patients.

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## P03.10

### Differential gene expression in chronic obstructive pulmonary disease cases and controls

**M. Soler Artigas**<sup>1,2</sup>, A. Esteve<sup>2</sup>, G. Thun<sup>2</sup>, C. Newby<sup>3</sup>, L. V. Wain<sup>1</sup>, M. D. Tobin<sup>1</sup>, S. Heath<sup>2</sup>, I. Gut<sup>2</sup>, D. Singh<sup>4</sup>, L. Ziegler-Heitbrock<sup>5</sup>, C. Brightling<sup>3</sup>;

<sup>1</sup>Genetic Epidemiology Group, Department of Health Sciences, University of Leicester, Leicester, United Kingdom, <sup>2</sup>Centro Nacional de Análisis Genómico (CNAG-CRG), Center for Genomic Regulation, Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>Department of Infection, Inflammation and Immunity, Institute for Lung Health, University of Leicester, Leicester, United Kingdom, <sup>4</sup>University of Manchester, Medicines Evaluation Unit Limited, Manchester, United Kingdom, <sup>5</sup>The EvA Study Center, Comprehensive Pneumology Center, Helmholtz-Zentrum München, Gauting, Germany.

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. However, the biological mechanisms underlying the development and progression of COPD are not yet well understood. We analysed sequence-based transcriptomics data in COPD cases and controls from the EvA study, as part of the AirPROM project. Cell material was obtained by airway epithelial brushing (305 cases/232 controls) and bronchoalveolar lavage (182 cases/174 controls).

Case-control differential expression was tested using an exact permutation test, and lasso regression was used to assess whether differentially expressed genes were able to predict case-control status. The dataset was divided into training and testing sets of randomly selected individuals, and average predictive values across 50 iterations of ~80% for brushing and ~70% for lavage were obtained. A small number of genes were selected in more than 40 out of the 50 iterations suggesting their potential relevance in biological processes relevant to COPD. Additionally, genes were clustered into modules according to their co-expression across individuals, and the association of these modules with COPD status and other related traits were tested using weighted gene co-expression network analysis. This approach highlighted some of the genes selected by the lasso regression analysis. Genome-wide data has now been generated for this set of individuals and integrative analysis is in progress.

Overall, these analyses will highlight genes relevant to biological processes present in COPD patients and have the potential to improve our understanding of this complex disease.

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## P03.11

### Targeted NGS improves the identification of genetic determinants in Combined Pituitary Hormone Deficiency and Septo-Optic Dysplasia

**J. Rodríguez-Contreras**<sup>1</sup>, C. Polo-Vaquero<sup>1</sup>, A. Del Pozo<sup>1,2</sup>, K. Ibáñez-Garikano<sup>1</sup>, J. Sillat<sup>1</sup>, V. E. F. Montano<sup>1</sup>, I. González-Casado<sup>3</sup>, B. Lecumberri-Santamaría<sup>4</sup>, P. D. Lapunzina<sup>1,2</sup>, E. Vallespín<sup>1,2</sup>, K. E. Heath<sup>1,2</sup>, A. Campos-Barros<sup>1,2</sup>;

<sup>1</sup>INGEMM, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>CIBERER (U753), ISCIII, Madrid, Spain, <sup>3</sup>Pediatric Endocrinology, Hospital Universitario La Paz, Madrid, Spain, <sup>4</sup>Endocrinology, Hospital Universitario La Paz, Madrid, Spain.

**Introduction:** Molecular diagnosis of combined pituitary hormone deficiency (CPHD) and septo-optic dysplasia (SOD) by classical gene screening cascade has a poor success rate due to the increasing number of implicated genes, the genetic overlap with other clinical entities such as congenital hypogonadotropic hypogonadism (CHH) and the recently postulated oligogenic inheritance.

**Aim:** To design, implement and validate a targeted NGS panel to identify the genetic determinants of CPHD and SOD.

#### Subjects and Methods

A cohort of 82 CPHD or SOD patients were analysed with the HYPOPIT.V1 panel, including a total of 73 genes: 50 found to be mutated in pituitary disorders and 23 genes in associated signalling pathways. Variants were validated by Sanger sequencing, MLPA or aCGH.

**Results:** After having completed the analysis of 26 patients (CPHD n=19; SOD, n=7), we have identified likely pathogenic variants in 11 (42%); 7 of which presented potentially relevant variants in more than one gene, some of them in genes known to be implicated in CHH, such as *FGFR1*, *FGF8*, and *PROKR2*, and in holoprosencephaly associated genes (*CDON*, *GLI2*, *ZIC2*, *PTCH1*, *EYA4*). Interestingly, in 3/11 patients, we also detected variants in *CHD7*, a gene implicated in CHARGE syndrome.

**Conclusions:** Targeted NGS identified disease relevant variants in 42% of analyzed CPHD and SOD cases, supporting the hypothesis of digenic/oligogenic inheritance in 27% of the examined patients. These results confirm the utility of the targeted NGS approach for improving the molecular diagnosis of CPHD and SOD.

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## P03.12

### Genetic variants underlying congenital cystic adenomatoid malformations

**M. Garcia-Barcelo**<sup>1</sup>, J. S. Hsu<sup>1</sup>, R. Zhang<sup>2</sup>, F. Yeung<sup>1</sup>, C. Wong<sup>1</sup>, M. Yu<sup>1</sup>, M. So<sup>1</sup>, M. Li<sup>1</sup>, P. Sham<sup>1</sup>, H. Xia<sup>2</sup>, P. K. Tam<sup>1</sup>;

<sup>1</sup>The University of Hong Kong, Hong Kong, Hong Kong, <sup>2</sup>Guangzhou Women and Children's Medical Center, Guangzhou, China.

**Introduction:** A congenital cystic adenomatoid malformation (CCAM) is a congenital hamartomatous pulmonary lesion that results from abnormal overgrowth of tracheal, bronchial, bronchiolar, or alveolar tissue. The clinical presentation is variable, ranging from the most serious phenotype which may result in hydrops fetalis to mild phenotype with possible resolution. Affected new-borns present with severe respiratory distress or remain asymptomatic until later in life. Surgical resection is the definitive treatment. The disorder is rare (1/8300-1/35,000 live births) and occurs mainly sporadically. The condition is due to a defective branching morphogenesis of the lung at different developmental stages. What triggers this developmental defect is unknown, yet alteration of the signalling pathways controlling lung development are thought to be the cause.

**Methods:** Whole exome sequencing was conducted on 15 CCAM trios following the standard protocol.

**Results:** Damaging de novo mutations in four genes known to be involved in airway epithelium response to cigarette smoking and lung cancer (*SPG11*, *MICAL2* and *LRRC16A*) or soft tissue sarcoma (*SCYL1*) found in 4/15 patients. Importantly, damaging mutations forming compound heterozygotes were detected in *SLC15A1*, a gene involved in lung peptide clearance in bronchial airway epithelial cell lines. Likewise, mutations in interacting genes co-existed in several patients and its relevance is being assessed. Copy number analysis is underway.

**Conclusion:** CCAM is genetically heterogeneous with different mutated genes in different patients and it is likely to require mutations in more than one gene to give rise to the developmental anomaly.

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## P03.13

**Integration of genomic analysis and transcript expression in focal form of congenital hyperinsulinism**I. Wieland<sup>1</sup>, I. Dallmann<sup>1</sup>, S. Vogelgesang<sup>2</sup>, W. Barthlen<sup>3</sup>, K. Mohnike<sup>4</sup>, M. Zenker<sup>1</sup>;<sup>1</sup>University Hospital, Institute of Human Genetics, Otto-von-Guericke University, Magdeburg, Germany, <sup>2</sup>Institute of Pathology, University Greifswald, Greifswald, Germany, <sup>3</sup>Clinic for Ped. Surgery, University Greifswald, Greifswald, Germany,<sup>4</sup>University Hospital, Dept. of Pediatrics, Otto-von-Guericke University, Magdeburg, Germany.

**Introduction:** In congenital hyperinsulinism (CHI) insulin secretion is dysregulated leading to severe hypoglycaemia in neonates and infants. Focal forms of CHI have been reported in 20-50% of mutation positive patients. The focal form is caused by a paternally transmitted recessive mutation in ABCC8 or KCNJ11 on chromosome 11p15 and a second somatic event in the affected islet of Langerhans. In pancreatic lesions we investigated the genetic mechanisms underlying focal form of CHI.

**Methods:** Patients with proven ABCC8 or KCNJ11 mutations and receiving therapeutic surgery were included. Loss of heterozygosity (LOH) and gene expression were analysed by PCR and Sanger sequencing. Deletions, duplications and uniparental isodisomy (UPD) were tested by methylation-specific MLPA. The effect of UPD on gene expression levels was analysed by massive analysis of cDNA ends (MACE) and real-time quantitative PCR.

**Results:** LOH was found in 10/11 focal lesions, in contrast to surrounding pancreatic tissue or blood cells. Monoallelic expression of mutant ABCC8/KCNJ11 alleles occurred in all lesions, whereas unaffected pancreatic tissue showed biallelic expression. Both, ABCC8 and KCNJ11, are located in proximity to the Beckwith-Wiedemann imprinting control region on chromosome 11p15 that is prone to UPD. Paternal UPD 11p15 was specifically found in samples showing LOH, neither deletion nor duplication was detected. Paternal UPD in focal lesions resulted in expression changes of 11p15 imprinted genes and upregulation of endocrine transcription factors.

**Conclusions:** Focal form of CHI is caused by somatic paternal UPD 11p15 leading to monoallelic expression of mutant ABCC8/KCNJ11 and affects gene expression of lesional endocrine cells.

## P03.14

**Molecular analysis of CYP21A2 gene in Argentine patients with Congenital Adrenal Hyperplasia**C. S. Fernandez<sup>1</sup>, M. Taboas<sup>2</sup>, C. D. Brueque<sup>1,3</sup>, N. Buzzalino<sup>1</sup>, L. Espeche<sup>1</sup>, M. Delea<sup>1</sup>, A. Nadra<sup>4</sup>, N. Ceballos<sup>4</sup>, L. Alba<sup>1</sup>, L. Dain<sup>1,3,4</sup>,<sup>1</sup>National Center of Medical Genetics, ANLIS, Ministry of Health, Buenos Aires, Argentina, <sup>2</sup>Regenerative Medicine Laboratory, Department of Orthopaedics and Traumatology, Juan Fernandez Hospital, Buenos Aires, Argentina, <sup>3</sup>Institute of Biology and Experimental Medicine, CONICET, Buenos Aires, Argentina, <sup>4</sup>School of Sciences, University of Buenos Aires, Buenos Aires, Argentina.

**Introduction:** Congenital adrenal hyperplasia, the most frequent inborn metabolism error, is caused in 90-95% of the cases, by mutations in the 21-hydroxylase gene (CYP21A2). The deficiency can present as severe, or classical form (C), and non-classical one (NC). CYP21A2 and C4 genes constitute the RCCX module. About 70% of haplotypes have a bimodular arrangement, with one module carrying CYP21A2 and the other the highly homologous pseudogene CYP21A1P. A regulatory region of the CYP21A2, named Z promoter (ZP), is located 5.6 kb upstream. Materials and Methods: 628 patients (137 C, 491 NC) diagnosed as 21-hydroxylase deficient were studied. In 380/628 we studied the most frequent mutations. In 248/628 the entire CYP21A2 gene was sequenced. Gene dosage was analyzed by MLPA. In 80/628 the ZP was sequenced. The RCCX region was studied by Southern blot, MLPA and long range PCR. *In vitro* and/or *in silico* analyses, developed in our laboratory, were performed to determine the biological implications of novel mutations. Results: 100% of C and 86% of NC patients were fully genotyped. We have identified 10 novel mutations in the coding region and one in the ZP. All novel variants showed pathogenicity. We identified 3 novel haplotypes among 16 different conformations of the RCCX module. Conclusions: The CYP21A2 gene is located in one of the most complex regions of the genome. Our results represent one of the larger series of patients in Latin America. We demonstrated the presence of novel mutations and haplotypes found for the first time in patients from our population.

## P03.15

**Evaluation of human epididymis protein 4 as a novel serum inflammatory biomarker in cystic fibrosis**B. Nagy Jr.<sup>1</sup>, L. Fila<sup>2</sup>, L. A. Clarke<sup>3</sup>, Z. Fejes<sup>1</sup>, P. Antal-Szalmás<sup>1</sup>, J. Kappelmayer<sup>4</sup>, M. Amaral<sup>3</sup>, M. Macek Jr.<sup>2</sup>, I. Balogh<sup>1</sup>,<sup>1</sup>University of Debrecen, Debrecen, Hungary, <sup>2</sup>Charles University, Prague, Czech Republic,<sup>3</sup>University of Lisboa, Lisboa, Portugal.

**Introduction:** A biomarker that can be used for the evaluation of disease progression and the treatment efficacy in cystic fibrosis (CF) would be of a great importance. Increased expression of the human epididymis protein 4 (HE4) was previously described in CF lung biopsies, but it is unknown whether serum HE4 concentrations are elevated in CF.

**Materials and Methods:** 77 young and 57 adult CF patients were enrolled together with 117 normal controls. Serum HE4 was measured by an immunoassay and its expression was investigated using RT-qPCR in CF versus non-CF respiratory epithelium biopsies. The expression of the potential regulator miR-140-5p was analyzed using an UPL-based RT-qPCR assay. HE4 was measured in the supernatants from unpolarized and polarized CF bronchial epithelial CFBE cells expressing wt- or F508del-CFTR.

**Results:** Serum HE4 levels were significantly elevated in affected children and in adult CF patients (median 99.5 and 115.7 pmol/L, respectively) compared to controls (36.3 pmol/L). In CF patients, HE4 levels positively correlated with disease severity and CRP concentrations, while an inverse relationship was found between HE4 and the spirometric FEV1 value. HE4 mRNA levels were significantly upregulated with a decreased miR-140-5p expression in the CF airway biopsies. There were 2-fold higher HE4 concentrations in the supernatant of polarized F508del-CFTR CFBE cells compared to wt cells.

**Conclusions:** HE4 serum levels correlate with the overall severity of CF and the degree of pulmonary dysfunction. HE4 may thus be utilized as novel inflammatory biomarker and possibly also as measure of treatment efficacy in CF lung disease.

## P03.16

**Genes underlying inherited cystic kidney disease in Arabian fetuses and neonates**M. H. Al-Hamed<sup>1</sup>, W. Kurdi<sup>1</sup>, M. Tulbah<sup>1</sup>, N. Alsahan<sup>1</sup>, Q. Ambosaidi<sup>1</sup>, J. Sayer<sup>2</sup>, B. Meyer<sup>1</sup>,<sup>1</sup>King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia, <sup>2</sup>Newcastle University, Newcastle upon Tyne, United Kingdom.

**Introduction:** Inherited cystic kidney disorders are a common cause of end-stage renal disease. These diseases are common in the Saudi population and are associated with significant morbidity and mortality. Over 50 ciliopathy genes, which encode proteins that influence the structure and function of the primary cilia, are implicated in cystic kidney disease.

**Materials and Methods:** In order to define the phenotype and genotype of cystic kidney disease in fetuses and neonates, we correlated antenatal ultrasound examination and postnatal renal ultrasound examination with targeted exon sequencing, using a renal gene panel. A cohort of 50 families in whom antenatal renal USS findings in affected cases included either bilateral cystic kidney disease, echogenic kidneys or enlarged kidneys was investigated.

**Results:** In this cohort, causative mutations and inferred causative mutations using parental DNA were detected in 35 families (70%). Extra renal malformations, including encephalocele, polydactyly and heart malformations, consistent with ciliopathy phenotypes, were frequently detected. Mutations were found in 14 different genes with a total of 15 novel pathogenic variants. Mutations in CC2D2A followed by PKHD1 were the most common cause of an antenatally suspected ciliopathy in our cohort.

**Conclusion:** In families with ciliopathy phenotypes, mutational analysis using a targeted gene panel allows a rapid molecular diagnosis and provides important information for patients, parents and their physicians.

## P03.17

**Hypothyroidism during fetal life impaired carbohydrate metabolism in young and aged offspring rat: Role of glucose transporters in insulin-sensitive tissue**H. Gholami<sup>1</sup>, S. Jedd<sup>2</sup>, F. Rouhollah<sup>1</sup>, M. Zarkesh<sup>3</sup>, A. Zadeh-Vakili<sup>3</sup>, A. Ghasemi<sup>2</sup>,<sup>1</sup>Department of Biology, Tehran Medical Branch, Islamic Azad University, Tehran, Iran,<sup>2</sup>Iran, Tehran, Islamic Republic of, <sup>3</sup>Endocrine Physiology Research Center and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University<sup>4</sup>of Medical Sciences, Tehran, Iran, Tehran, Islamic Republic of, <sup>5</sup>Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of.

**Introduction:** Both thyroid hormone deficiency during fetal life and aging decreases insulin secretion capacity and lead to glucose intolerance in the

later life; the underlying mechanisms however are unknown. This study aimed to determine whether fetal hypothyroidism (FH) affects GLUT1 and GLUT4 gene expressions in insulin-sensitive tissue of young (3 months) and aged (19 months) offspring rats.

**Materials and Methods:** Pregnant Wistar rats were divided into two groups: The FH group received water containing 0.025% 6-propyl-2-thiouracil during gestation and the controls consumed tap water. Offspring from both groups (n=6 in each group) were followed until month 3 and month 19. GLUT1 and GLUT4 mRNA expression in isolated heart, adipose tissue, and soleus muscle were measured using real-time polymerase chain reaction in 3 and 19 months offspring.

**Results:** Compared with controls, in FH rats, GLUT1 expression was lower in all tissues in both the young (34%, 41% and 84% for heart, adipose and soleus, respectively) and aged rats (51%, 64% and 78% for heart, adipose and soleus, respectively). In addition, FH rats had lower expression of GLUT4 in adipose tissue (84% for young and 88% for aged rats) and higher expression in heart (104% for young and 388% for aged rats) and soleus (290% for young and 591% for aged rats) tissues.

**Conclusion:** Hypothyroidism during fetal life affects mRNA expression of glucose transporters during later life in both young and aged rats; these changes may contribute to impaired carbohydrate metabolism.

### P03.18

#### Novel mutations in the MODY genes HNF4 $\alpha$ , GCK, HNF1 $\alpha$ in a Castilla y Leon population

*M. De Pedro Muñoz<sup>1,2</sup>, M. Fernández Prieto<sup>1,2</sup>, E. Ruiz Pérez<sup>2</sup>, F. Gómez Peralta<sup>4</sup>, G. Díaz Soto<sup>3</sup>, J. Garrote Adrados<sup>6</sup>, R. Aguado García<sup>7</sup>, J. Miralles García<sup>8</sup>, R. González Sarmiento<sup>1,2</sup>;*

<sup>1</sup>Unidad de Medicina Molecular, Departamento de Medicina. Facultad de Medicina. Campus Miguel de Unamuno, Salamanca, Spain, <sup>2</sup>Instituto de investigación biomédica de Salamanca (IBSAL), Salamanca, Spain, <sup>3</sup>Servicio de Endocrinología y Nutrición, hospital militar de Burgos, Burgos, Spain, <sup>4</sup>Servicio de Endocrinología y Nutrición, hospital general de Segovia, Segovia, Spain, <sup>5</sup>Servicio de Endocrinología y Nutrición, hospital clínico universitario de Valladolid, Valladolid, Spain, <sup>6</sup>Servicio de Análisis Clínicos, hospital universitario Rio Hortega, Valladolid, Spain, <sup>7</sup>Servicio de Endocrinología y Nutrición, hospital universitario de León, León, Spain, <sup>8</sup>Servicio de Endocrinología y Nutrición, hospital universitario de Salamanca, Salamanca, Spain.

**Introduction:** Maturity onset diabetes of the young (MODY) is a rare monogenic form of diabetes mellitus. This disease is characterized by autosomal dominant mode of inheritance and an early onset being frequently diagnosed before 25 years of age. The aim of this study was to identify novel mutations in the most prevalent MODY genes (HNF4 $\alpha$ , GCK and HNF1 $\alpha$ ) in patients from Castilla y Leon.

**Patients and Methods:** We have studied 232 patients with clinical diagnosis of MODY from Hospitals from Castilla y Leon. The HNF4 $\alpha$ , GCK and HNF1 $\alpha$  genes were analyzed by direct sequencing. In silico predictions of the pathogenicity were carried out using: PolyPhen, pMut and SIFT.

**Results:** We have identified a total of 46 patients with mutations in HNF4 $\alpha$ , GCK or HNF1 $\alpha$  genes (20%) and 15 from these were novel mutations (32,60%). A missense mutation and a nonsense mutation had not been previously reported in the HNF4 $\alpha$  gene (p.T117I, p.R131X), both in exon 4. The following novel variations were identified in GCK gene: IVS7+1G>A, p.M57I, p.T116P, p.K205N, p.Trp257\_Leu266delinsPLLLLfsX27, p.F316fsX352, p.C371X, p.V374M, p.G385A, p.Y413X. Three more unreported mutations were identified in HNF1 $\alpha$  gene (p.T137fsX17, c.865 INSC, p.T458I). In silico analysis predicted all of the novel mutations to be pathogenic.

**Conclusions:** Recurrent mutations have not been detected in our cohort of MODY patients. It implies that there is still a high incidence of novel mutations. It is necessary to study all the coding region and adjacent intronic regions of prevalent MODY genes for improving the diagnosis.

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### P03.19

#### A new submicroscopic duplication of the 8q24.3 region is a potential candidate for disorders of sex development

*F. N. Dilek<sup>1,2</sup>, F. E. Perçin<sup>1</sup>, H. Kayserili<sup>3,4</sup>, M. A. Ergün<sup>1</sup>, N. Saka<sup>5</sup>;*

<sup>1</sup>Gazi University School of Medicine, Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Diyarbakır Maternity and Child Hospital, Diyarbakır, Turkey, <sup>3</sup>Istanbul Medical Faculty of Istanbul University, Department of Pediatrics & Medical Genetics, Istanbul, Turkey, <sup>4</sup>Koç University School of Medicine, Department of Medical Genetics, Istanbul, Turkey, <sup>5</sup>Istanbul University Faculty of Medicine, Department of Pediatric Endocrinology, Istanbul, Turkey.

**Introduction:** Some of the disorders of sex development (DSD), including 46,XX testicular DSD formerly called "XX maleness" and 46,XY DSD with partial or complete gonadal dysgenesis primarily affect the gonads. but can also result in non-syndromic XY-GD. The causative mutations in known ge-

nes have not been identified in more than 50% of 46,XY DSD cases. Among the detectable causative factors, deletions or nucleotide mutations of the SRY gene account for about 10-15 %. This implies that the vast majority of patients with gonadal DSD do not have an underlying molecular etiopathogenesis as of yet.

**Material and methods:** Ten unrelated patients diagnosed as complete 46,XY GD were selected to be included in the study. All patients with the diagnosis of XY complete GD met the clinical criteria for the inclusion. Sequence analysis of SRY, WT1 and CBX2 genes were performed in all patients and the genomic DNA from 10 unrelated females with complete 46,XY GD were processed on the Affymetrix Cyto2.7M array consistent with the manufacturer's protocol.

**Results:** The analysis result suggested that the most significant region maps to chromosome 8q24.3 which was previously reported by another independent study with a similar patient cohort and this region being probable candidate related to complete 46,XY GD.

**Conclusion:** Duplication of 8q24.3 region may be responsible for DSD etiology, because of in total three different cases with either partial or complete gonadal dysgenesis have been identified.

This study was funded by the Gazi University Scientific Research Foundation.

### P03.20

#### Hemolytic uremic syndrome in children-our experience

*M. Gafencu<sup>1</sup>, A. Lungu<sup>2</sup>, O. Limoncu<sup>2</sup>, C. Stoica<sup>2</sup>, Z. Prohaszka<sup>3</sup>, A. Jianu<sup>4</sup>, M. Puiu<sup>1</sup>, G. Doros<sup>1</sup>;*

<sup>1</sup>Emergency Children Hospital, Victor Babes University of medicine and Pharmacy, Timisoara, Romania, <sup>2</sup>Fundeni Clinical Institute, Bucuresti, Romania, <sup>3</sup>Semmelweis University, 3rd Department of Medicine, Research Laboratory, Budapest, Hungary,

<sup>4</sup>University of Medicine & Pharmacy, Timisoara, Roumania, Timisoara, Romania.

**Aims.** Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia with fragmented erythrocytes, thrombocytopenia and acute renal failure. The aim of the study is to present the renal replacement therapy methods in our groups, genetic changes linked with the type of HUS, complications and mortality rate.

**Methods.** A retrospective analysis was supported on 49 children patients, aged 2 months to 17 years, with the diagnosis of HUS. We analyze the cases using the classic classification and evaluate the cases using data linked to renal replacement therapy need and mortality. Genetic testing was performed by Semmelweis University Budapest for 3 aHUS patients.

**Results.** More than 80% of patients were dialyzed. Mortality was correlated with the severity of extra renal involvement (less than 20%). One patient was found to be homozygous for a common deletion of CFHR1 and CFHR3 genes, no other copy number alterations were identified (although in parents). The second patient was found to be heterozygous for a substitution in exon 23 of the CFH gene (c.3592G>C) causing a glutamic acid to glutamine change at codon 1198 of the complement factor H protein (p.E1198Q) and heterozygous for a substitution in exon 7 of the CFHR5 gene (c.1067G>A). Other patient was found to be heterozygous for a mutation causing amino acid change (Q950H) in scr16 of complement factor H, and heterozygous for the rare allele of the CFH E936D polymorphism.

**Conclusions.** Different mutations were discovered in our cases, some expected to have a pathogenic role in the development of aHUS.

### P03.21

#### Genetic testing in hypogonadotropic hypogonadism using next generation sequencing

*K. Trebušák Podkrajšek<sup>1,2</sup>, T. Obreza<sup>1</sup>, M. Pfeifer<sup>3</sup>, A. Dankovčíková<sup>4</sup>, J. Kovač<sup>1</sup>, T. Battelino<sup>1,2</sup>, M. Avbelj Stefanija<sup>1</sup>;*

<sup>1</sup>University medical centre Ljubljana, University Children's Hospital, Ljubljana, Slovenia, <sup>2</sup>University of Ljubljana, Faculty of medicine, Ljubljana, Slovenia, <sup>3</sup>University medical centre Ljubljana, Division of internal medicine, Ljubljana, Slovenia, <sup>4</sup>University children's hospital, Kosice, Slovakia.

**Introduction:** Congenital hypogonadotropic hypogonadism (HH) is a rare but clinically and genetically heterogeneous disease caused by pathogenic mutations in several genes leading to impaired production and secretion of gonadotropin hormones and consequently sex hormones. HH is characterized by an absence or delay of puberty and infertility. The association of HH with hyposmia or anosmia is defined as Kallmann syndrome. Molecular genetic testing of HH is important, as it can prompt the treatment.

**Materials and Methods:** 11 subjects (10 males, one female) aged between 16 and 67 years with suspicion of congenital HH were included. Seven males had Kallmann syndrome, 3 males had normosmic HH. The female had suspicion of Kallmann syndrome. Three subjects had other congenital anomalies associated with HH (colour blindness, anomalies of heart, learning

disabilities, long limbs, sensorineural deafness, bimanual synkinesis, hypertelorism). Targeted next generation sequencing (NGS) of 24 genes known to be associated with HH was used to identify genetic variants that were subsequently confirmed by Sanger sequencing.

Results: Six mutations in five genes (PROK2, GNRHR, PROKR2, FGFR1 and CHD7) were detected in six patients. Among them, three variants namely PROK2 NM\_001126128.1: c.171\_172delTT (p.Ile57MetfsTer17), FGFR1 NM\_023110.2: c.196T>C (p.Tyr66Arg) and CHD7 NM\_017780.3: c.5759A>G (p.Tyr1920Cys) have not yet been described.

Conclusion: NGS enables fast and reliable identification of causal mutations in several genes related to HH simultaneously. Presented subject group with HH was genetically very diverse and the results expand the spectrum of mutations implicated in HH.

### P03.22

#### Clinical utility of a kidney disease gene panel for genetic diagnosis of cystic and glomerular inherited kidney diseases

G. Bullrich<sup>1</sup>, I. Vargas<sup>1</sup>, P. Ruiz<sup>1</sup>, L. Lorente<sup>1</sup>, D. Trujillano<sup>2</sup>, X. Estivill<sup>2</sup>, J. Ballarín<sup>1</sup>, R. Torra<sup>1</sup>, E. Ars<sup>1</sup>;

<sup>1</sup>Fundació Puigvert, Barcelona, Spain, <sup>2</sup>Centre for Genomic Regulation, Barcelona, Spain.

Introduction: Molecular diagnosis of cystic and glomerular inherited kidney diseases (IKD) is complicated by their high genetic heterogeneity and phenotypic variability. Clinical manifestations of these diseases cover a broad range of phenotypes that can be mimicked by mutations in several genes. We aimed to develop a more comprehensive approach for genetic diagnosis of these IKD.

Materials and methods: Massive parallel sequencing of 124 genes causative or associated with cystic or glomerular IKD was performed in 216 patients, a validation cohort (114 patients) and a diagnostic cohort (102 patients).

Results: We identified 136 of the 140 mutations in the validation cohort including several CNVs, demonstrating similar sensitivity than Sanger sequencing with the advantage of detecting CNVs in a single approach. Moreover, the kidney panel allows a more comprehensive analysis by identifying 1) mutations in low-frequency mutated genes, such as *NPHP3* mutations in patients with clinical suspicion of autosomal recessive polycystic kidney disease and *CUBN* mutations in nephrotic syndrome; 2) complex inheritance patterns, such as autosomal dominant polycystic kidney disease patients with two pathogenic mutations in *PKD1* and/or *PKD2* genes or Alport syndrome patients with mutations in two different collagen IV genes; 3) coinheritance of mutations in two genes causative of different IKD.

Conclusions: Massive parallel sequencing of this kidney-disease gene panel is a comprehensive, efficient and cost-effective tool for genetic diagnosis of cystic and glomerular IKD.

### P03.23

#### Targeted NGS panel improves mutation detection rate in congenital hypogonadotropic hypogonadism and Kallmann syndrome patients and confirms the implication of further genes in these pathologies

C. Polo-Vaquero<sup>1</sup>, J. Rodriguez-Contreras<sup>1</sup>, M. Aza-Carmona<sup>1,2</sup>, E. Vallespin<sup>1,2</sup>, K. Ibañez-Garikano<sup>1</sup>, J. Silla<sup>1</sup>, P. Lapunzina<sup>1,2</sup>, B. Lecumberri-Santamaría<sup>1</sup>, I. González-Casado<sup>3</sup>, A. Campos-Barros<sup>1</sup>, K. E. Heath<sup>1,2</sup>;

<sup>1</sup>Institute of Medical & Molecular Genetics, Hospital Universitario La Paz, Madrid, Spain,

<sup>2</sup>CIBERER, ISCIII, Madrid, Spain, <sup>3</sup>Dept. Pediatric Endocrinology, Hospital Universitario La Paz, Madrid, Spain.

Introduction: The large number of implicated genes and their oligogenic inheritance complicates the genetic diagnosis of patients with congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome (KS).

Aim: We set out to identify the molecular defect in a cohort of CHH, HH and KS patients using a targeted NGS panel. Subsequently we evaluated the number of cases with monogenic or oligogenic inheritance.

Methods. Cohort of 58 probands: 39 CHH, 16 KS, 2 CHARGE with HH and 1 CAH with HH. All were screened for mutations using the NGS panel, HYPOPIT.V1, which includes a total of 73 genes: 50 found to be mutated in pituitary disorders and 23 genes in associated signalling pathways. Variants were validated by Sanger sequencing, MLPA or arrayCGH.

Results: Pathogenic or likely pathogenic mutations were identified in 19/58 probands (33%). The phenotype could be explained by the identified mutations in 8 of these (42%). In the remaining 11 probands, only one mutation was identified; 9 in genes with reported oligogenicity, and two are individuals with heterozygous variants in GNRHR and GNRH1 suggesting the possibility of oligogenic inheritance for these genes. Interestingly, a heterozygous mutation in POU1F1 was observed in an adult patient with KS.

Conclusions: Targeted NGS has increased our mutation detection rate and has also demonstrated the implication of further genes in the etiology of these pathologies.

### P03.24

#### Fetal kidney anomalies: Next generation sequencing

M. Rasmussen<sup>1</sup>, L. Sunde<sup>1,2</sup>, M. L. Nielsen<sup>1</sup>, M. Ramsing<sup>3</sup>, A. Petersen<sup>4</sup>, T. D. Hjortshøj<sup>5</sup>, T. E. Olsen<sup>5</sup>, A. Tabor<sup>6</sup>, J. M. Hertz<sup>7</sup>, I. Johnsen<sup>8</sup>, L. S. Sperling<sup>9</sup>, O. B. Petersen<sup>10</sup>, U. B. Jensen<sup>1,2</sup>, M. B. Petersen<sup>11</sup>, D. L. Lildballe<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark,

<sup>2</sup>Department of Biomedicine, Aarhus, Denmark, <sup>3</sup>Department of Pathology, Randers Regional Hospital, Randers, Denmark, <sup>4</sup>Department of Pathology, Aalborg University Hospital, Aalborg, Denmark, <sup>5</sup>Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark, <sup>6</sup>Department of Obstetrics, Rigshospitalet, Copenhagen, Denmark, <sup>7</sup>Department of Clinical Genetics, Odense University Hospital, Odense, Denmark, <sup>8</sup>Department of Pathology, Odense University Hospital, Odense, Denmark,

<sup>9</sup>Department of Gynaecology and Obstetrics, Odense University Hospital, Odense, Denmark, <sup>10</sup>Department of Gynaecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark, <sup>11</sup>Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark.

Aim and Introduction: Identification of abnormal kidneys in the fetus may lead to termination of the pregnancy and raises questions about the underlying cause and recurrence risk in future pregnancies.

In this study, we investigate the effectiveness of targeted next generation sequencing in fetuses with prenatally detected kidney anomalies in order to uncover genetic explanations and assess recurrence risk. Also, we aim to study the relation between genetic findings and post mortem kidney histology.

Methods: The study comprises fetuses diagnosed prenatally with bilateral kidney anomalies that have undergone postmortem examination.

The approximately 110 genes included in the targeted panel were chosen on the basis of their potential involvement in embryonic kidney development, cystic kidney disease, or the renin-angiotensin system.

DNA was extracted from fetal tissue samples or cultured chorion villus cells, aminocytes, or fibroblasts. Data analysis was performed using CLC Genomics workbench, publicly available databases, and prediction tools.

The Regional Ethical Committee approved the study.

Results: Samples from 61 fetuses from 57 families were included in the study.

Two fetuses had mutations in the nephronophthisis associated gene, TMEM67 and six fetuses had mutations in kidney developmental genes. For these fetuses kidney histology is presented.

Conclusion and Perspectives: In eight (14%) fetuses we identified a likely genetic cause of the kidney anomalies.

Ten fetuses from eight families, in which no mutations were identified, have been selected for exome sequencing in order to uncover novel genes associated to fetal kidney anomalies.

### P03.25

#### Development of a comprehensive workflow for analysis of LPL gene, identification of LPLD patients eligible to receive AAV1- LPLS447X gene therapy.

I. Valaskova<sup>1,2</sup>, J. Krizova<sup>1,3</sup>, R. Gaillyova<sup>1,2</sup>;

<sup>1</sup>University Hospital, Brno, Czech Republic, <sup>2</sup>Masaryk University, Brno, Czech Republic,

<sup>3</sup>Mendel University, Brno, Czech Republic.

Lipoprotein lipase (LPL) deficiency (LPLD) is characterized by severe hypertriglyceridaemia. The most debilitating clinical manifestation of the disease is recurrent, severe and potentially life-threatening acute pancreatitis. LPLD is caused by loss-of-function mutations in the gene encoding LPL, regulated the plasma levels of triglyceride. Variant of the *LPL* gene Ser447stop called *LPL<sup>S447X</sup>* is the only variant associated with increased LPL enzyme activity leading to reduced triglyceride levels.

It was developed a LPL gene therapy product, Alipogene tiparvovec (Glybera, AMT-01, AAV1- LPLS447X), an adeno-associated virus serotype 1-based gene therapy that results in the expression of gain-of-function S447X variant of the human LPL gene.

The aim of this work was to establish a molecular genetic screening program of patients with LPLD caused by loss-of-function mutations in LPL gene, suitable for AAV1- LPLS447X gene therapy.

Method: The examined group has consisted of patients with severe or multiple pancreatitis attacks. Genomic DNA has been extracted from whole blood. Screening algorithm in LPLD patients has been: 1. HRM-based LightSNIp assay to detect the most common LPL mutation Asn291Ser (cooperation with TIBMolBiol), 2. MLPA (P218 MRC Holand) confirmed using QF PCR, 3. sequencing LPL promoter and 10 exons.

Results: In LPLD patients, synonymous and nonsynonymous LPL sequence variants have been detected. In silico analysis have been performed to determine the pathological status of sequence variants with uncertain significance.

Conclusion: It is currently possible to identify patients with LPLD caused by loss-of-function mutations in LPL gene, eligible for the introduction of treatment of the therapeutic *LPL<sup>S447X</sup>* gene.

**P03.26****Crohn's disease associated with a higher rate of mevalonate kinase somatic mosaicism in circulating monocytes.**

*Y. Shinar<sup>1</sup>, R. Cohen<sup>1</sup>, V. Marcu-Malina<sup>1</sup>, I. Goldstein<sup>1</sup>, A. Menza-Vilaro<sup>2</sup>, A. Menza-Vilaro<sup>2</sup>, E. Gonzalez-Roca<sup>2</sup>, J. I. Arostegui<sup>2</sup>, A. Livneh<sup>1,3</sup>, V. Oron-Karni<sup>4</sup>;*

<sup>1</sup>Sheba Medical Center, Ramat Gan, Israel, <sup>2</sup>Hospital Clinic, Barcelona, Spain, <sup>3</sup>Te Aviv University, Tel Aviv, Israel, <sup>4</sup>Rhenium LTD, Modi'in, Israel.

**Introduction** The role of somatic mutations in the development of complex autoinflammatory diseases has barely been studied. We performed genetic testing for familiar monogenic autoinflammatory diseases in the case of an adult with late onset recurrent fever and severe upper abdominal pain. The patient was eventually clinically diagnosed with Crohn's disease based on capsule endoscopy and was treated successfully with an anti-TNF blocker.

**Aim** Study the rate and distribution of a novel and somatic mevalonate kinase (MVK) gene mutation detected by Sanger sequencing, in a patient with Crohn's disease.

**Methods** Massive parallel sequencing (MPS) of all MVK exons (2000 reads of target) was performed on DNA isolated from peripheral blood cells, and followed by exon 3 fusion-PCR MPS (10,000 reads per target) in DNA from neutrophils, and from FACS sorted T (CD3+), B (CD19+) and monocyte (CD14+) cells.

**Results** The somatic mutation was detected in 22% of the MVK exon 3 reads and was confirmed to be the only mutation in the entire MVK coding region. The mosaicism rate was twice higher in DNA of CD14+ monocytes (50%) than in neutrophils (23%), B (17.6%) or T cells (21.7%).

**Conclusion** This may be the first report of a somatic MVK mutation in the context of Crohn's disease. The mutation source was traced as far as a multi-potential hematopoietic stem cell. However the mutation appeared to expand in circulating CD14+ monocytes despite effective anti-TNF treatment. The pathogenic and auto-inflammatory significance of this monocyte sub-population remains to be studied.

**P03.27****Rapid screening for mutations in MODY genes by next generation sequencing**

*M. Losekoot<sup>1</sup>, L. Vijfhuizen<sup>1</sup>, W. S. Frankhuizen<sup>1</sup>, C. M. J. Spruit<sup>1</sup>, A. H. B. Bollen<sup>1</sup>, I. F. H. C. Fokkema<sup>1</sup>, N. van der Stoep<sup>1</sup>;*

<sup>1</sup>Department of Clinical Genetics, Leiden, Netherlands, <sup>2</sup>Department of Human Genetics, Leiden, Netherlands.

**Introduction:** Maturity onset diabetes of the young (MODY) is a monogenic form of diabetes with an autosomal dominant pattern of inheritance. It is characterized by early onset (<25 years of age), non-obese and non-auto-immune mediated associated diabetes and accounts for 1-5% of all diabetic patients. Differentiation of the various subtypes allows an optimal treatment and delay of diabetes related complications. Mutations in at least 13 genes are responsible for MODY and therefore mutation analysis of all genes in one next generation sequencing experiment seems an optimal diagnostic strategy.

**Materials en Methods:** All coding exons and at least 20 bp of flanking intronic sequences were enriched using the Agilent SureSelectXT Inherited Disease Panel, followed by sequencing on Illumina HiSeq2000. Data analysis was performed using a home-made pipeline for MODY related genes.

**Results:** High-quality sequence data was obtained. Variant analysis using a low stringency analysis pipeline revealed a pathogenic mutation in 10 out of 69 patients in GCK, HNF1A, HNF4A or HNF1B. In addition, 4 possibly pathogenic mutations were found in ABCC8 and KLF11, genes that are not routinely tested in MODY patients.

**Conclusions:** This approach gives a mutation detection ratio comparable to Sanger Sequencing. The procedure is fast and efficient and reveals mutations in genes that otherwise might not have been tested in a gene-by gene approach.

**P03.28****Variable clinical expressivity in patients with HNF1B mutations**

*A. Tranchina<sup>1</sup>, C. Graziano<sup>1</sup>, R. Minardi<sup>2,3</sup>, C. P. Cristalli<sup>2,3</sup>, S. Bin<sup>3</sup>, A. Mattiaccio<sup>3</sup>, M. Parial<sup>3</sup>, G. Lanzoni<sup>1</sup>, M. Romagnoli<sup>4</sup>, G. Severi<sup>1</sup>, G. La Manna<sup>2</sup>, M. Seri<sup>1</sup>, V. Mantovani<sup>1,3</sup>;*

<sup>1</sup>Medical Genetics Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy,

<sup>2</sup>Nephrology Dialysis and Transplantation Unit, S.Orsola-Malpighi University Hospital, Bologna, Italy, <sup>3</sup>Center for Applied Biomedical Research (CRBA), S.Orsola-Malpighi University Hospital, Bologna, Italy.

HNF1B heterozygous mutations are the cause of "Renal Cysts and Diabetes Syndrome", a clinically heterogeneous disorder characterized by maturity onset diabetes of the young, renal disease (cysts, small kidney, hyperuricemic nephropathy), liver test anomalies (cholestasis) and abnormalities of the genital tract.

In the last 4 years, we tested 29 patients for a suspected HNF1B-related disorder. Through sequencing and MLPA analysis, we identified a mutation in 14 patients from 11 unrelated families. Six patients carried large deletions involving the whole gene (de novo in three patients with available parental DNA). One carried an intragenic deletion (exon 1 to 4). One had a splicing mutation. One had a promoter variant of uncertain significance. Five patients from 2 unrelated families carried missense mutations.

No typical clinical presentation can be described. Patients with loss of function mutations (gene deletions and splicing) tend to have an earlier onset of symptoms, with hyperechogenic kidneys detected in the antenatal period and congenital urinary and/or genital malformation. Progression of kidney disease appears to be slow. All adult patients had diabetes mellitus or glucose intolerance. Two had cholestasis and two developed unexplained liver cirrhosis at a relatively young age (39 and 44 years). A 21-year-old woman also had mild developmental delay: after MLPA analysis that identified an HNF1B deletion, array-CGH was performed and a large (1.3 Mb) deletion was found.

In conclusion, we confirm the wide phenotypic heterogeneity of HNF1B-related disorders, but we point out that liver disease can be a major complication in patients.

**P03.29****Whole-exome sequencing (WES) identifies rare mutations in two pedigrees with recurrent familial Nephrolithiasis**

*M. Jabalameli, V. Walker, S. Ennis;*

*University of Southampton, Southampton, United Kingdom.*

**Introduction:** Nephrolithiasis is a prevalent condition with high morbidity. Heritability accounts for 56% of the disease incidence. Patients often present with acute flank pain and dysuria that necessitates surgical intervention. High concordance rate among monozygotic twins suggest a strong genetic component. Recent studies on monogenic cause of nephrolithiasis identified that dominant mutations are more frequent in adult onset kidney stones. We sought to identify pathogenic mutations underlying late-onset nephrolithiasis in two pedigrees with recurrent stone formers.

**Materials and Methods:** 6 adults from two pedigrees with multiple stone formers were recruited. Biochemical markers of renal function were comprehensively analysed and genetic diagnosis carried out by whole exome sequencing.

**Results:** Based on segregation pattern, deleteriousness (Phylop and Gerp++ scores) and scarcity of variant (MAF≤0.02) both in the reference population and in the in-house database, two missense mutations in *ADCY10*(p.P1200L) and *SLC25A25*(p.Q349H) were identified to underlie the condition in pedigree 1 and 2 respectively.

**Conclusion:** *ADCY10* encodes a unique class of adenylyl cyclases. Variation at this gene has been already proposed to underlie absorptive hypercalciuria. We identified a rare missense mutation in exon 26 of *ADCY10* (c.C3599T) that co-segregate with nephrolithiasis in pedigree-1. Furthermore, WES analysis revealed that a missense mutation in exon 8 of *SLC25A25* (c.G1047C) co-segregate with nephrolithiasis in pedigree-2. *SLC25A25* encodes a calcium-binding carrier that regulates uptake or efflux of adenine nucleotides into or from mitochondria. Our in-silico analysis suggested that variants involved in  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  metabolism and purine metabolism contribute to hereditary nephrolithiasis and their functional impact merit further investigation.

**P03.30****Whole exome sequencing identifies a novel mutation in ACTN4 gene in a Brazilian patient with steroid resistant nephrotic syndrome**

*M. S. Guaragna, A. Lutaif, A. Maciel-Guerra, V. Belanger, L. Prates, G. Guerra-Júnior, M. de Mello;*

*UNICAMP, Campinas, Brazil.*

**Introduction:** Nephrotic syndrome (NS) is defined by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Approximately 20% of the patients do not respond to treatment with steroids and are classified as steroid-resistant (SRNS). A significant number of SRNS cases are caused by inherited mutations in proteins that form the glomerular filtration barrier. The aim of this study was to search for mutations in a SRNS familial case, using whole exome sequencing (WES), as this method has become a tool of choice for genetic screening in diseases with high genetic heterogeneity and phenotypic variability such as SRNS.

**Material and Methods:** WES was performed in one familial SRNS case. *NPHS1*, *NPHS2* and *WT1* mutations had been already excluded after Sanger sequencing. We used strict genetic criteria for reduction of variants. Sanger sequencing was performed in order to confirm the mutation identified.

**Results:** We identified the novel heterozygous p.Phe153Leu mutation in the *ACTN4* gene in the patient and in his father, who is also affected with the disease. The residue Phe153 localizes in the N-terminal region of  $\alpha$ -actinin-4 protein and is conserved down to *Danio Rerio*. Mutations in the N-terminal region are described as increasing the affinity to actin filaments and affecting their intracellular localization.

**Conclusions:** We identified a novel mutation in *ACTN4*. The patient here presented and his family will benefit of the unequivocal etiology of the disease and of genetic counseling, as mutations in *ACTN4* are associated with late onset autosomal-dominant familial FSGS.

FAPESP and CNPq supported this work.

### P03.31

#### Extensive molecular analysis suggested the strong genetic heterogeneity of idiopathic chronic pancreatitis

*V. Sofia<sup>1</sup>, L. Da Sacco<sup>2</sup>, C. Surace<sup>1</sup>, A. Tomaiuolo<sup>1</sup>, S. Genovese<sup>3</sup>, S. Grotta<sup>3</sup>, M. Gnazzo<sup>1</sup>, S. Petrocchi<sup>1</sup>, L. Ciocca<sup>4</sup>, F. Alghisi<sup>4</sup>, E. Montemirto<sup>4</sup>, L. Martemucci<sup>5</sup>, A. Elce<sup>6</sup>, V. Lucidi<sup>1</sup>, G. Castaldo<sup>7</sup>, A. Novelli<sup>8</sup>, A. Angioni<sup>1</sup>;*

<sup>1</sup>Laboratory of Medical Genetics, "Bambino Gesù" Children's Hospital, Rome, Italy,

<sup>2</sup>Multifactorial Diseases and Complex Phenotypes Research Area, "Bambino Gesù" Children's Hospital, Rome, Italy, <sup>3</sup>Laboratory of Medical Genetics, "Bambino Gesù" Children's Hospital/S. Pietro Fatebenefratelli Hospital, UOSD Medical Genetics, Rome, Italy, <sup>4</sup>Cystic Fibrosis Unit, "Bambino Gesù" Children's Hospital, Rome, Italy, <sup>5</sup>Pediatria Sistematica, A.O. Santobono-Pausilipon, Naples, Italy, <sup>6</sup>Ceinge-Biotecnologie avanzate, Naples, Italy, <sup>7</sup>Ceinge-Biotecnologie avanzate/Dipartimento di Medicina Molecolare and Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy.

**Introduction:** Genetic features of Chronic Pancreatitis (CP) have been extensively investigated mainly testing genes associated to the trypsinogen activation pathway. However, different molecular pathways involving other genes may be implicated in the pathogenesis of CP.

**Materials and Methods:** 81 patients with Idiopathic CP have been investigated using Next Generation Sequencing approach with a panel of 70 genes related to six different pancreatic pathways: premature activation of trypsinogen; modifier genes of Cystic Fibrosis phenotype; pancreatic secretion and ion homeostasis; Calcium signalling and zymogen granules exocytosis; autophagy; autoimmune pancreatitis related genes.

**Results:** We found mutations in 34 out of 70 genes examined. 65/81 patients (80.2%) were positive for mutations in one or more genes, 16/81 patients (19.8%) had no mutations. Mutations in the *CFTR* (Cystic fibrosis trans-membrane conductance regulator) gene were detected in 33/81 patients (40.7%) and 23 of them exhibited at least one mutation in genes of other pancreatic pathways. Of the remaining 48 patients, 13/81 (16%) had mutations in genes involved in premature activation of trypsinogen and 19/81 (23.5%) had mutations only in genes of the other pathways: 40/65 patients positives for mutations showed variants in two or more genes (61.5%).

**Conclusions:** Our data suggest a high rate of genetic heterogeneity in chronic pancreatitis and that trans-heterozygosity may predispose to the Idiopathic CP phenotype.

### P03.32

#### Genetic diagnosis by specific sequencing of PKD1 gene in a (spanish) cohort of 100 ADPKD families

*M. Molero, C. Ruiz, A. Gomez, A. Arteche, L. Rausell, M. Lazaro, M. Bermejo, J. Garcia-Planells, M. Garcia-Hoyos;*  
*Instituto de Medicina Genómica (IMEGEN), Paterna, Spain.*

Autosomal dominant polycystic kidney disease (ADPKD; # 173900 and #613095) is the most common inherited kidney disease and is characterized by the development and progressive enlargement of cysts in kidneys and other organs, eventually leading to end-stage renal disease and usually a late-onset multisystem disorder characterized by bilateral renal cysts. ADPKD is caused by mutations in the PKD1 gene (\*601313) in approximately 85% of the cases and in the PKD2 gene (\*173910) in the remaining 15%. The genetic diagnosis of this disease is very difficult due to the existence of 5 pseudogenes with high resemblance (98%) to the PKD1 gene.

We have developed, validated, and carried out a methodology based on a long PCR followed by several internal PCRs that allows specific and accurate analysis of the entire PKD1 gene while avoiding the amplification of the pseudogenes.

We used this long PCR approach for the analysis of the PKD1 gene in more than 100 ADPKD families. This procedure allowed the identification of the disease-causing mutation in 75.3% of our patients. The vast majority of the mutations detected are null variants (nonsense, frameshift, and canonical splice sites).

According to the results produced in this study, a high percentage of ADPKD cases is associated with mutations in the PKD1 gene. Specific sequencing of

the PKD1 is the most efficient approach to determine the molecular cause of the disease in ADPKD patients. An accurate genetic diagnosis is essential to provide reproductive options to ADPKD patients and their families in addition to appropriate genetic counselling.

### P03.33

#### Molecular characterization of genetic variants in patients with primary ciliary dyskinesia from Serbia

*M. Z. Andjelkovic<sup>1</sup>, V. Spasovski<sup>1</sup>, M. Vreca<sup>1</sup>, P. Minic<sup>2,3</sup>, A. Sovtic<sup>2</sup>, M. Rodic<sup>2</sup>, S. Pavlovic<sup>1</sup>;*

<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, <sup>2</sup>Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic“, Belgrade, Serbia, <sup>3</sup>School of Medicine, University of Belgrade, Belgrade, Serbia.

Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive disorder with extensive genetic heterogeneity and is estimated to affect 1 in 15,000 to 30,000 individuals. It usually comes to medical attention because of recurrent pneumonia, otitis media and upper respiratory tract infections early in the life, or, in the adult male, infertility. Respiratory infections are caused by defective mucociliary clearance due to immotile or dysmotile respiratory cilia with or without ultrastructural defects.

In this study, we analyzed 6 patients from Serbia with diagnosis of PCD according to clinical presentation. We used a NGS panel with 4813 genes to detect disease-causing mutations in these patients. We have detected variants in 6 different genes. In each patient only one gene was affected. Variants were detected in the genes previously associated with PCD: *DNAL1* (c.347A>T, c.350T>G and c.485G>A), *LRRK6* (c.27T>G and c.1397T>C), *DNAH11* (c.7798C>T, c.8555C>G), *DNAH5* (c.1137C>T, c.7624T>C, c.4356-2A>G) and *CCDC40* (c. [248delC]; [248delC]), from first to the fifth patient, respectively. In the last patient we detected homozygous variant in *SCNN1A* gene (c. [1654T>C]; [1654T>C]). So far, *SCNN1A* gene was not associated with PCD, but it was associated with Cystic Fibrosis-Like Disease.

This study provided the first data about molecular genetics of patients from Serbia presenting with PCD clinical symptoms, thus paving a path to molecular genetic diagnostics and genetic counseling of this disease in the country. Acknowledgements

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### P03.34

#### The prevalence of GNAS deficiency-related diseases in a large cohort of patients characterized by the EuroPHP network

*F. M. Elli<sup>1</sup>, A. Linglart<sup>2,3</sup>, I. Garin<sup>4</sup>, L. de Sanctis<sup>5</sup>, P. Bordogna<sup>1</sup>, V. Grybek<sup>2,3</sup>, A. Pereda<sup>4</sup>, F. Giachero<sup>5</sup>, E. Verrua<sup>1</sup>, P. Hanna<sup>3,2</sup>, G. Mantovani<sup>6</sup>, G. Perez de Nanclares<sup>4</sup>;*

<sup>1</sup>Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Milan, Italy, <sup>2</sup>APHP, Reference center for rare disorders of the mineral metabolism and Plateforme d'expertise Paris Sud Maladies Rares, Le Kremlin Bicêtre, France,

<sup>3</sup>INSERM U1169, Hôpital Bicêtre, Le Kremlin Bicêtre, et Université Paris-Saclay, Le Kremlin Bicêtre, France, <sup>4</sup>Hospital Universitario Araba, Vitoria-Gasteiz, Spain,

<sup>5</sup>Department of Public Health and Pediatrics, University of Turin, Regina Margherita Children's Hospital, Health and Science City, Turin, Italy.

**Context:** The term pseudohypoparathyroidism (PHP) was coined to describe the clinical condition resulting from end-organ resistance to parathyroid hormone (rPTH), caused by (epi)genetic alterations within or upstream GNAS. Though knowledge is growing, there are few data on the prevalence of underlying (epi)genetic defects.

**Objective:** To ascertain the relative prevalence of PHP-associated molecular defects.

**Design:** With a special questionnaire, we collected data from patients (n=407) clinically and molecularly characterized by the EuroPHP network.

**Results:** Isolated rPTH (31%) was caused only by epigenetic defects, 70% of patients showing loss of imprinting affecting all four GNAS differentially methylated regions (DMRs) and 30% loss of methylation restricted to the GNAS A/B:TSS-DMR. Multihormone resistance without Albright's hereditary osteodystrophy (AHO) (15%) was essentially due to epigenetic defects, although 10% had point mutations.

In patients with rPTH and AHO (10%), the rate of point mutations was higher (28%) and methylation defects lower (70%). Patients with multihormone resistance and AHO (38%), presented all types of molecular defects with different frequencies. Finally, isolated AHO (4%) and progressive osseous heteroplasia (2%) were exclusively caused by point mutations.

**Conclusion:** We have established the prevalence of various genetic and epigenetic lesions in PHP-affected patients. Using these findings, we will develop criteria to guide cost-effective strategies for genetic testing and explore the implications for management and prognosis.

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### P03.35

#### Acute pancreatitis recurrence transcriptome profiling in chylomicronemia

*K. Tremblay, D. Brisson, D. Gaudet;*

*Université de Montréal and ECOGENE-21 Research Center, Chicoutimi, QC, Canada.*

**Introduction:** Recurrent acute pancreatitis (RAP) is defined as more than two crisis of acute pancreatitis not meeting the criteria of chronic pancreatitis. Extreme hypertriglyceridemia (fasting triglycerides >10 mmol/L) is associated with chylomicronemia (CM) and increased risk of RAP. The recurrence of pancreatitis episodes varies significantly between CM patients. We have investigated the gene expression profiles of adult patients with CM in a spectrum of RAP.

**Materials and Methods:** A total of 62 subjects participated in this study: 15 healthy controls and 47 CM subjects divided into three groups: 0 (n=21), 1-3 (n=10) or ≥4 (n=16) acute pancreatitis episodes. Whole blood RNA samples were hybridized on Affymetrix Human Gene 2.0 ST Array. After RMA normalization, differential expression moderated T-tests (Bioconductor package Limma) and Ingenuity Pathway Analyses were performed.

**Results:** At a p-value <0.01, a FDR of 5% (Benjamini-Hochberg method) and a >2-fold change expression significance levels, a set of 41 probes have been found differentially expressed in CM subjects with no pancreatitis, 103 in the CM group with 1 to 3 pancreatitis, and 94 in the group with ≥4 pancreatitis compared to controls. Of the identified annotated probes, 14 are shared by all CM groups; 3 are specific to CM with no pancreatitis; 11 are specific to CM with 1 to 3 pancreatitis, and 17 are specific to CM with ≥4 pancreatitis. Most of the annotated probes are involved in inflammatory, immune, lipoprotein kinetics or signalling biological pathways.

**Conclusions:** These results reveal gene expression signatures of RAP in patients with CM.

### P03.36

#### Two novel RFX6 variants in siblings with Mitchell-Riley syndrome with childhood onset diabetes

*M. Skopkova<sup>1</sup>, M. Ciljakova<sup>2</sup>, Z. Havlicekova<sup>2</sup>, J. Vojtikova<sup>2</sup>, L. Valentinova<sup>1</sup>, D. Danis<sup>1</sup>, D. Murgas<sup>3</sup>, R. Szepesova<sup>2</sup>, J. Stanik<sup>1,4,5</sup>, P. Banovcik<sup>2</sup>, I. Klimes<sup>1</sup>, D. Gasperikova<sup>1</sup>;*

<sup>1</sup>*Institute of Experimental Endocrinology BMC SAS, Bratislava, Slovakia, <sup>2</sup>Department of Children and Adolescents, University Hospital in Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia, <sup>3</sup>Department of Pediatric Surgery, University Hospital in Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia, <sup>4</sup>First Department of Pediatrics, Medical Faculty of Comenius University and Children Faculty Hospital, Bratislava, Slovakia, <sup>5</sup>Center for Pediatric Research, Hospital for Children & Adolescents, University of Leipzig, Leipzig, Germany.*

**Introduction:** Two sisters from a Caucasian family were diagnosed with multiple gastrointestinal tract (GI) malformations (duodenal atresia, Meckel diverticulum, gallbladder hypoplasia, and multiple gastric heterotopy loci in ileum) and childhood onset diabetes (in 2y 10m and 2y 7m). The aim of our study was to search for the genetic etiology.

**Materials and Methods:** The whole exome (SureSelect V4+UTR, Agilent) sequencing was performed (HiSeq2000, Illumina) in the two patients and their parents. Variant filtering included MAF<0.01, autosomal recessive mode of inheritance conditions and pedigree analysis. Candidate variants were confirmed by Sanger sequencing and their pathogenicity was tested by in silico analyses.

**Results:** In both affected sisters, two novel heterozygous RFX6 variants were identified: c.1316\_1319delTCTA (p.I439Tfs\*13) and c.1154G>A (p.R385Q). Each was inherited from another unaffected parent. Both variants were found to be pathogenic by in silico analyses. Mutations in the gene RFX6 are associated with neonatal diabetes, pancreatic and biliary hypoplasia and duodenal/jejunal atresia - classified as the Mitchell-Riley syndrome (MRS) with autosomal recessive type of inheritance.

**Conclusions:** From 11 previously reported patients with MRS, all but two of them developed diabetes in first days of life. We report here two sisters with two new compound heterozygous RFX6 variants with phenotypes consistent with MRS, but with diabetes onset in the third year of life. This supports the need of extending the diabetes definition in MRS to „neonatal or childhood“ onset and suggests that glycaemia should regularly be checked in patients with GI tract malformations typical for Mitchell-Riley syndrome. Supported by APVV 0187-12.

### P03.37

#### Role of *Klotho* genetic polymorphisms in salt-sensitivity: a link between salt and aging?

*L. Citterio<sup>1</sup>, C. Lanzani<sup>1</sup>, S. Delli Carpini<sup>1</sup>, S. Lupoli<sup>2</sup>, E. Brioni<sup>1</sup>, M. Simonini<sup>1</sup>, G. Gatti<sup>1</sup>, S. Tentori<sup>1</sup>, L. Zagato<sup>1</sup>, E. Messaggio<sup>1</sup>, C. Barlassina<sup>2</sup>, D. Cusi<sup>2</sup>, P. Manunta<sup>1</sup>;*

<sup>1</sup>*University "Vita-Salute", Milano, Italy, <sup>2</sup>Department of Health Science, University of Milan, Milano, Italy.*

**Rational:** Previous data in transgenic mice showed that one-half *Klotho* deficiency resulted in extensive premature aging, increased salt sensitivity and caused salt-sensitive hypertension. Moreover, a recent gene expression study confirmed the expression of *Klotho* in renal distal tubular cells. Aim of the study: To evaluate the role of *Klotho* polymorphisms in human essential hypertension and salt sensitivity.

**Design and Methods:** 32 SNPs in *Klotho* gene identified with a previous GWA were used in the genetic analysis. Selected SNPs were studied in three different clinical settings: 1. Pressure-natriuresis relationship (PNat) in 580 naive essential hypertensive patients, never treated before, (NHP) by Acute salt load (NaLoad: 310 mMol in 2 h iv), 2. Low salt diet (137 pts, Low SD: <100 mEq/die for 15 days), and 3. Follow-up cohort (FUP, median 150 months) of 230 hypertensive patients.

**Results:** 15 SNPs (with tagging  $r^2=0.80$ ) out of 32 were selected in the identified gene region. Six of these resulted significantly associated to BP variation after Naload and/or LowSD and/or FUP. Particularly, the genotypes in a missense mutation in exon 2 were associated to salt sensitivity (SBP variation and PNat after Na load) and decrease in kidney function in FUP confirming the similar effect in the two different clinical settings.

**Conclusion:** Selected *Klotho* gene SNPs for first time resulted involved in salt homeostasis, hypertension development and long term kidney damage. Our findings support the proposed *Klotho* as key gene in salt sensitivity and aging in humans.

### P03.38

#### Computational strategy to predict pathogenic variants in SERPINA1 gene led to the identification of new functional mutations

*E. Giacopuzzi, M. Laffranchi, R. Berardelli, G. Borsani, A. Fra;*

*Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.*

**Introduction:** The SERPINA1 gene encodes for Alpha-1-Antitrypsin (AAT), an acute phase glycoprotein that inhibits Neutrophil Elastase (NHE). Mutations in this gene cause reduced levels of AAT in the plasma, Alpha-1 Antitrypsin Deficiency (AATD), and reduced inhibition of NHE leads to emphysema. Moreover, mutated forms of AAT can aggregate in the endoplasmic reticulum of hepatocytes causing hepatotoxicity and liver disease. AATD is characterized by high allelic heterogeneity, asking for a way to discriminate functionally impaired alleles as new missense variants are observed in the gene.

**Methods:** By combining CADD score, amino acid conservation, protein structure modeling and alignment with other SERPIN polypeptides, we have developed a computational strategy to identify missense variations that could result in impaired protein function. We tested our strategy against the group of already characterized pathogenic missense mutations to assess specificity and sensibility of the method. This approach has then been applied to missense variants reported in human population from the ExAc 0.3 database (~63.000 exomes) to identify candidate missense mutations for functional studies.

**Results:** Our strategy performed well to correctly classify already known pathogenic mutations. When applied to ExAc 0.3 data, it identified 8 yet uncharacterized missense variants with high probability to affect the SERPINA1 protein function. These variants could represent new rare pathogenic alleles diffused in the human population. Functional consequences of these mutations are being evaluated by in vitro studies.

**Conclusions:** We have developed a computational strategy useful to prioritize new pathogenic variants in the SERPINA1 gene and identify new potential pathogenic alleles.

### P03.39

#### Disease exome, a powerful diagnostic tool: post-mortem diagnosis of dyskeratosis congenita

*J. Pinto-Basto<sup>1</sup>, L. S. Almeida<sup>1</sup>, F. Melo<sup>1</sup>, A. Garrido<sup>2</sup>, C. Barbosa<sup>2</sup>, S. M. Pereira<sup>1</sup>, R. Cerqueira<sup>1</sup>, P. Tavares<sup>1</sup>;*

<sup>1</sup>*CGC Genetics, Porto, Portugal, <sup>2</sup>Hospital Center Tondela – Viseu, E.P.E, Viseu, Portugal.*

Exome sequencing has become a powerful diagnostic tool to identify the molecular etiology of genetic diseases. We report on a deceased 60yo male with an undiagnosed systemic disease that presented with liver cirrhosis, pulmonary fibrosis, sick sinus syndrome and thrombocytopenia. DNA from post-mortem tissue was obtained and disease exome performed.

Disease exome was performed by capture of target regions using TruSightO-ne (Illumina) and subsequent NGS of a panel composed by 4813 clinically-relevant genes. Alignment and variant calling was performed using the BWA and GATK, respectively. Variants were filtered and processed with bioinformatic analysis tools to assess its pathogenicity and potential to explain the clinical phenotype. Relevant variants were confirmed by Sanger sequencing. Analysis revealed a novel missense heterozygous variant c.1492G>A (p.Gly498Arg) in TERT gene, affecting a highly conserved residue. Bioinformatic analysis suggests that the variant is very likely pathogenic and is not present in dbSNP, ExAC, 1KGenomes and ESP. Mutations in TERT gene, mostly missense, cause autosomal dominant dyskeratosis congenita (DKC; type2; MIM 613989) and autosomal recessive DKC (MIM 613989). In the context of the clinical phenotype of this patient, this result supports the diagnosis of DKC type2. Segregation studies were not possible as parents and sibs were not available for testing.

We report a post-mortem diagnosis of DKC type2 with a novel variant in the TERT gene, expanding the mutational spectrum of TERT related DKC. Additionally, this result highlights the importance of molecular diagnosis, even post-mortem, as establishing the molecular etiology allows proper genetic counselling to at-risk relatives.

#### P03.40

##### Sequence analysis of EPCAM gene in Consanguineous Qatari Families, suffering from tufting enteropathy, identified a founder mutation

Z. Nawaz<sup>1</sup>, M. A. Khan<sup>2</sup>, S. Khan<sup>2</sup>, S. Ali<sup>1</sup>, H. Abdelrahman<sup>3</sup>, E. abdalbari hamid<sup>3</sup>, F. Al-madhaki<sup>2</sup>, W. Almasri<sup>2</sup>, K. Abu Hasima<sup>2</sup>, K. Hassan<sup>3</sup>;

<sup>1</sup>Genomic Diagnostic Division, Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Doha, Qatar; <sup>2</sup>Genomic Core Facility, Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar;

<sup>3</sup>Department of Pediatric, Hamad General Hospital, Hamad Medical Corporation, Doha, Qatar.

**Background:** Tufting enteropathy, also termed as intestinal epithelial dysplasia, is a rare genetic condition with early onset of sever diarrhea. Histologically, it is characterized by villous atrophy, disorganization of the surface epithelium and basement membrane aberrations. The epidemiological data indicates its prevalence as 1 out of 50,000 to 100,000 live births in western population, while it is estimated to be greater in Arab ethnic families due to high rate of consanguinity. Genetic studies have found the association of EPCAM and SPINT2 genes mutation in disease etiology.

**Materials and Methods:** Sanger DNA sequencing was carried out for the genetic screening of EPCAM gene in affected and corresponding normal family members. The study was approved by ethical committee and sample was obtained after informed written consent.

**Results:** Two consanguineous Qatari families were enrolled in the present study and sanger sequencing of all coding exons in EPCAM gene was performed. The sequence analysis revealed c.498\_499insC mutation in exon 5, which presumably truncates the protein synthesis (p.Gln167Profs\*21). This truncation predictably removes the C-terminal domain of EPCAM protein, which is suggestively involved in the attachment of EPCAM protein to intracellular membrane.

**Conclusion:** The identified mutation, c.498\_499insC, seems to have founder effect in Arab population because it has previously been reported in a Qatari, Kuwaiti and Saudi families. The present molecular study has evidenced this mutation as a genetic hotspot and suggests to formulate a molecular diagnostic test in Qatari families, affected with tufting enteropathy, for genetic counseling.

#### P03.41

##### Is COMMD1 gene a modifier locus of Wilson disease?

A. Zarina<sup>1,2</sup>, L. Piekuse<sup>1</sup>, M. Kreile<sup>1,2</sup>, I. Tolmane<sup>2,4</sup>, V. Sondore<sup>3</sup>, G. Cernevskas<sup>4</sup>, I. Pukite<sup>5</sup>, Z. Krumina<sup>5</sup>;

<sup>1</sup>Riga Stradiņš University Scientific Laboratory of Molecular Genetics, Riga, Latvia; <sup>2</sup>Riga

Stradiņš University, Department of Biology and Microbiology, Riga, Latvia; <sup>3</sup>Riga East

Clinical University Hospital, stationary "Infectology Center of Latvia", Riga, Latvia;

<sup>4</sup>University of Latvia, Faculty of Medicine, Riga, Latvia; <sup>5</sup>Children Clinical university

Hospital, Riga, Latvia.

**Introduction.** Wilson disease (WD) is an autosomal recessive disorder of copper metabolism, caused by mutations in gene ATP7B. Although WD is established as monogenic disorder, heterogeneity in phenotype is observed even among patients possessing the same type of mutations in gene ATP7B. One of the candidate genes which might play the role of a modifier locus is COMMD1 (former MURR1).

**Materials and methods.** 54 patients, clinically diagnosed with WD (Leipzig's Score, 2001) were tested for mutations in ATP7B gene: by PCR Bi-PASA and direct sequencing (for other mutations); and for possible changes in gene COMMD1 by direct sequencing of its three exons.

Results. Wilson disease was genetically confirmed in 42 patients. In total 82.4% of WD patients' alleles were identified: H1069Q (72.22%); M769Hfs\*26 (1.85%); Val73GlufsX4 (1.85%) and D1267G, D765N, M645R (0.93% for each); 27.78% of the alleles remained unidentified. Three nucleotide changes were identified in COMMD1, of which two represented reported synonymous changes: rs55677935 and rs9096. The third one – two nucleotide deletion in gene's regulatory region (rs569267407) was found only in two patients – siblings, showing different clinical symptoms – one dying from fulminant hepatitis at age of 17, the second – being asymptomatic at age of 19, when first being diagnosed with WD due to family screening.

#### Conclusions.

- 1) There were no variants in gene COMMD1 found that could be related to different phenotype of WD patients;
- 2) Change found in COMMD1 regulatory part could be a cause for different protein activity, but the clinical significance remains unclear.

## P04 Skeletal, connective tissue, ectodermal and skin disorders

#### P04.001

##### OBSL1 Mutations Represent The Major Gene Defect In A Group Of 3M Syndrome Patients: A Study From Turkey

P. O. Simsek-Kiper<sup>1</sup>, E. Z. Taskiran<sup>2</sup>, U. Arslan<sup>3</sup>, C. Kosukcu<sup>2</sup>, O. Akgun-Dogan<sup>1</sup>, G. E. Utine<sup>1</sup>, A. Alkasifoglu<sup>4</sup>, N. Kandemir<sup>4</sup>, V. Cormier-Daire<sup>5</sup>, Y. Alanay<sup>6</sup>, M. Alkasifoglu<sup>2</sup>, K. Boduroglu<sup>1</sup>,

<sup>1</sup>Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Genetics, Ankara, Turkey; <sup>2</sup>Hacettepe University, Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey; <sup>3</sup>Hacettepe University, Institute of Public Health, Department of Epidemiology, Ankara, Turkey; <sup>4</sup>Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Ankara, Turkey; <sup>5</sup>INSERM U781, Université Paris Descartes-Sorbonne Paris Cite, Institut Imagine, Hôpital Necker Enfants Malades Genetics, Paris, France; <sup>6</sup>Acıbadem University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Genetics, Istanbul, Turkey.

**Introduction:** 3M syndrome is characterized by severe prenatal and postnatal growth retardation, facial features and normal intelligence. Mutations in CUL7, OBSL1 or CCDC8 genes have been identified in the etiology so far, CUL7 mutations being the most common. **Materials and Methods:** Clinical and molecular features of 18 patients from 14 families were evaluated. DNA sequence analysis of CUL7, OBSL1 and CCDC8 was performed and genotype-phenotype correlations were investigated. **Results:** Eight distinct CUL7 or OBSL1 homozygous mutations were identified in 11 patients. Of mutation positive patients (n=11), 6 (55%) had OBSL1 and 5 (45%) had CUL7 mutations. Of all detected mutations 1 CUL7 (c.361\_362insT) and 3 OBSL1 (c.1125\_1126insT, c.1277\_1282+delTCAAAGTCAG, c.1187G>A) mutations were novel. Growth hormone deficiency was detected in 1 patient with CUL7 and 2 patients with OBSL1 mutation. Birth weight of patients with CUL7 mutation was significantly lower than the patients with OBSL1 mutation (p=0.016). A group of patients with no mutations shared similar clinical and radiological features with mutation positive patients. **Conclusions:** 3M syndrome is a genetically heterogeneous condition. Although CUL7 was reported to be the major gene responsible for 3M syndrome so far, OBSL1 mutations were more common in the present study which might be an indicator of a founder effect in Turkish population. Mutations identified so far do not account for all 3M syndrome patients suggesting the involvement of other genes. Further molecular studies will shed light on the identification of these new genes in the common growth pathway.

#### P04.002

##### Altered ABCC9 gene: expanding the clinical phenotype of Cantu Syndrome

H. Fryssira<sup>1</sup>, E. Tsoutsou<sup>1</sup>, S. Psoni<sup>1</sup>, S. Amenta<sup>2</sup>, H. Lüdecke<sup>3</sup>, J. Czeschik<sup>3</sup>;

<sup>1</sup>Medical Genetics, School of Medicine, National and Kapodistrian University of Athens,

<sup>2</sup>"Aghia Sophia" Children's Hospital, Athens, Greece; <sup>3</sup>"Mittera" Maternity Hospital, Athens, Athens, Greece; <sup>3</sup>Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany.

**Introduction:** Cantu Syndrome (OMIM#239850) is a very rare (about fifty cases have been reported so far) autosomal dominant disorder characterized by generalized congenital hypertrichosis, neonatal macrosomia, distinctive coarse faces, cardiomegaly and skeletal abnormalities. The syndrome has been attributed to mutated ABCC9 and KCNJ8 genes which encode the SUR2 and Kir6.1 subunits of the ATP-dependent potassium channel (KATP-channel) respectively.

**Case Report:** We present a four-year-old female patient with developmental delay, dysmorphic features such as narrow forehead with low anterior frontal hairline, micrognathia, low-set dysplastic ears and generalized hypertrichosis since birth. The investigation revealed absent ovaries. FSH and PRL were markedly elevated whilst LH, estradiol, testosterone, DHEA-S and 17-OH progesterone were all normal. Chromosome G-banding technique was also normal. DNA sequencing analysis of the ABCC9 gene was performed and a heterozygous point mutation c.3460C>T which resulted in the abnormal protein NM\_005691.3 (ABCC9\_1001):p.Arg1154Trp was revealed. This missense, gain-of-function mutation was located in exon 27 of the ABCC9 gene.

**Conclusions:** The p.Arg1154Trp point mutation has been reported before in Cantu syndrome patients and is associated to the full phenotype. However, the absence of the ovaries has not been described before. Therefore, this could represent either an expansion of the phenotype or an interaction of ABCC9 with other genes related to ovarian formation and function.

Since the activating mutations in the ABCC9 gene are the underlying cause of the Cantu syndrome, a drug that leads to K<sup>+</sup> channel closure independently to ATP, could serve as a possible treatment.

#### P04.003

##### Acrodermatitis enteropathica-like forms of atopic dermatitis revealed by whole-exome sequencing

*S. Kury<sup>1</sup>, T. Besnard<sup>2</sup>, X. Latypova<sup>1</sup>, S. Schmitt<sup>1</sup>, S. P. Paul<sup>2</sup>, D. Candy<sup>3</sup>, E. Blouin<sup>4</sup>, S. Bézieau<sup>1</sup>;*

<sup>1</sup>CHU de Nantes, Nantes, France, <sup>2</sup>St Richard's Hospital, Chichester, United Kingdom,

<sup>3</sup>Institute of Child Health, London, United Kingdom, <sup>4</sup>Laboratoire Labcatal, Montrouge, France.

**Background:** Acrodermatitis enteropathica (AE; MIM #201100) is a rare and severe autosomal recessive zinc deficiency disorder molecularly diagnosed by the identification of a biallelic anomaly of zinc transporter gene SLC39A4. Its pathognomonic symptom is an acral and periorificial dermatitis occurring in early life that responds in a spectacular fashion to zinc therapy. Yet, our experience in the molecular diagnosis of AE showed that more than a half of the cases of suspected AE were not due to a primary zinc dyshomeostasis that would be associated by alterations of SLC39A4.

**Purpose:** Our goal was to determine the genetic cause of genodermatoses very suggestive of an AE, albeit with no SLC39A4 mutation.

**Method:** We sequenced the whole-exome of a series of simplex and multiplex families with a AE-like dermatitis.

**Results:** Beside the identification of a few exceedingly rare syndromes mimicking AE, the most striking result was the presence of dominant mutations in filaggrin and in laminins, some of them were already known to be associated with an increased susceptibility to atopic dermatitis (AD).

**Conclusions:** Our work highlighted atopic dermatitis as the main clinical diagnosis of AE. This suggests that a sub-group of the common disease represented by AD could be diagnosed by genetic testing. Further studies are needed to determine whether these severe cases of AD are exceptional or if the efficiency of zinc therapy can be extended to the treatment of all AD patients with similar anomalies.

#### P04.004

##### Functional modelling of SLC39A4 zinc transporter mutations effects in Acrodermatitis enteropathica

*X. Latypova<sup>1</sup>, T. Besnard<sup>2</sup>, E. Blouin<sup>2</sup>, P. Hulin<sup>3</sup>, S. Nedellec<sup>3</sup>, S. Bézieau<sup>1</sup>, S. Kury<sup>1</sup>;*

<sup>1</sup>Service de Génétique Médicale, CHU Nantes, Nantes, France, <sup>2</sup>Laboratoire LABCATAL, Montrouge, France, <sup>3</sup>Plateau d'imagerie cellulaire MicroPiCell, Nantes, France.

**Background:** Zinc is a key factor of various biological processes. Intestinal absorption is the critical step of zinc homeostasis regulation. *Acrodermatitis enteropathica* is a rare cause of hereditary zinc deficiency. Mutations in SLC39A4, coding a transmembrane zinc transporter, mainly expressed in enterocytes, have been described in *Acrodermatitis enteropathica* patients. Since its identification by our group in 2002, clinical molecular testing for SLC39A4 mutations is available. However, functional consequences of SLC39A4 mutations are poorly characterized. In these conditions, assessment of pathogenicity of newly identified variants of unknown significance remains challenging.

**Methods:** We evaluated the effects of missense SLC39A4 mutations in HeLa cells transfected with wild type or mutated, hemagglutinin tagged, SLC39A4 transcript. To model decrease of transport activity by SLC39A4, we performed time-lapse measurements of zinc cytoplasmic levels. Finally, the effects of putative splice-site mutations were tested by minigene assay.

**Results:** Disruption of SLC39A4 membrane trafficking has been showed in 8 missense mutations, located in N-terminal extracellular domain of the

transporter. Effect on splicing of c.192 G>C mutation of exon 1 of SLC39A4 has been confirmed. We propose a strategy to quantify zinc transport to investigate consequences of mutations without effect on SLC39A4 localization.

**Conclusion:** Functional assessment of SLC39A4 mutational spectrum provides key information for interpreting the significance of pathogenicity in the daily practice of a molecular biology laboratory.

#### P04.005

##### Broadening the phenotypic spectrum of heterozygous aggrecan mutations in short stature children

*O. Nilsson<sup>1</sup>, K. E. Heath<sup>2,3,4</sup>, M. Andrew<sup>5</sup>, L. Sentchordi<sup>6,7,8</sup>, S. Hallgrimsdottir<sup>5</sup>, A. Brannstrom<sup>5</sup>, M. Crocker<sup>1</sup>, J. Douglas<sup>9</sup>, N. Dunbar<sup>10</sup>, D. Flynn<sup>11</sup>, V. Hwa<sup>12</sup>, A. Jorge<sup>13</sup>, S. LaFranchi<sup>14</sup>, S. Lalani<sup>15</sup>, E. Los<sup>14</sup>, D. Newbern<sup>16</sup>, C. B. Nowak<sup>9</sup>, M. Olson<sup>16</sup>, J. Popovic<sup>17</sup>, J. Quintos<sup>18</sup>, E. Stattin<sup>19</sup>, J. Baron<sup>20</sup>, A. Dauber<sup>21</sup>, International Aggrecan Consortium; <sup>1</sup>Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>Institute of Medical & Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, UAM, Madrid, Spain, <sup>3</sup>Multidisciplinary Unit of Skeletal dysplasias (UMDE), Hospital Universitario La Paz, Madrid, Spain, <sup>4</sup>CIBERER, ISCIII, Madrid, Spain, <sup>5</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, <sup>6</sup>Institute of Medical & Molecular Genetics, IdiPAZ, Hospital Universitario La Paz, UAM, Madrid, Spain, <sup>7</sup>Hospital Universitario Infanta Leonor, Madrid, Spain, <sup>8</sup>Multidisciplinary Unit of Skeletal dysplasias (UMDE), Hospital Universitario La Paz, Madrid, Austria, <sup>9</sup>Boston Children's Hospital, Boston, MA, United States, <sup>10</sup>Connecticut Children's Hospital, Connecticut, CT, United States, <sup>11</sup>St. Luke Regional Medical Center, Boise, ID, United States, <sup>12</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, <sup>13</sup>University Sao Paulo, Sao Paulo, Brazil, <sup>14</sup>Oregon Health and Science University, Oregon, OR, United States, <sup>15</sup>Texas Children's Hospital, Texas, TX, United States, <sup>16</sup>Phoenix Children's Hospital, Phoenix, AZ, United States, <sup>17</sup>Children's Hospital of Pittsburgh, Pittsburgh, PA, United States, <sup>18</sup>Hasbro Children's Hospital, Rhode Island, RI, United States, <sup>19</sup>Umeå Universitet, Umeå, Sweden, <sup>20</sup>PDEGEN, NICHD, National Institute of Health, Bethesda, MD, United States, <sup>21</sup>Cincinnati Center for Growth Disorders, Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, ID, United States.*

**Introduction:** Heterozygous aggrecan (ACAN) mutations have been reported to cause short stature with bone age (BA) acceleration and premature growth cessation.

In order to characterize the phenotypic spectrum and the response to growth-promoting therapies, a detailed clinical evaluation of 66 individuals from 12 families with ACAN mutations, 7 novel truncations, 5 missense, was performed.

**Results:** Adults have mildly disproportionate short stature (SDS median:-3.3, range:-0.9 to -4.5) with normal/slightly elevated sitting height indices (SDS median:2.1, range:0.4-3.8) and early growth cessation and/or lack of pubertal growth spurt. Growth of upper extremities appears less affected with arm-spans typically greater than height (arm-span-height: median:+5.5cm; range:-4 to +13). Some cases had osteochondritis dissecans and early-onset osteoarthritis requiring knee joint and other large joints replacement surgeries in the 2nd-3rd decade. Two families had degenerative disc disease in the 4th decade.

Prepubertal height is less affected (median SDS:-1.8, range:-1.0 to -4.2). In contrast to most children with short stature, many with ACAN mutations show an advanced BA (BA-CA, median:+1.3yr; range:+0 to +3.5) reflecting a reduction in the remaining growth potential. Nine patients have been treated with rhGH and/or GnRH analogs or aromatase inhibitors.

**Conclusions:** Heterozygous ACAN mutations result in a wide phenotypic spectrum ranging from mild and proportionate short stature to a distinct skeletal dysplasia with disproportionate short stature and brachydactyly. Some also cause articular/intervertebral disc cartilage dysfunction leading to early-onset osteoarthritis and degenerative disc disease requiring intervention. Careful monitoring of patients with ACAN mutations may help to identify important genotype-phenotype correlations and to understand their pathogenicity.

#### P04.006

##### Analysis of PMN function in patients with aggressive periodontitis

*R. Werner<sup>1</sup>, A. Schossig<sup>1</sup>, J. Dudás<sup>2</sup>, J. Zschocke<sup>1</sup>, I. Kapferer-Seebacher<sup>3</sup>;*

<sup>1</sup>Division of Human Genetics, Medical University, Innsbruck, Austria, <sup>2</sup>Department of Otorhinolaryngology, Medical University, Innsbruck, Austria, <sup>3</sup>Department of Restorative and Operative Dentistry, Dental School, Medical University, Innsbruck, Austria.

**Introduction:** Aggressive periodontitis (AgP) is an inflammation disorder with a prevalence of approximately 0.1% in central European population. It is characterized by early age of onset, rapid rate of progression and loss of teeth until age of 35, if untreated. Several studies indicate functional changes in polymorphonuclear leucocytes (PMNs) resulting in altered immune response and destruction of periodontal tissues.

**Material and methods:** We have investigated phagocytosis and chemotaxis of PMNs extracted from peripheral blood in a 4-generation family with ge-

neralized autosomal dominant inherited AgP (6 affected, 5 non-affected, 5 unrelated controls) as well as in a cohort of unrelated patients with sporadic form of generalized AgP (n = 46) and periodontal healthy 1:2 age- and gender-matched controls.

**Results:** Phagocytic activity of PMNs in response to non-opsonized *E.coli* was significantly reduced in familial AgP patients compared to healthy siblings and non-related controls ( $p = 0.032$ ). A similar reduction of phagocytic activity could not be observed in sporadic AgP in the cohort compared to matched controls. In the family both directed and random migration of PMNs was significantly reduced in AgP patients compared to healthy controls ( $p = 0.042$  and  $p = 0.028$ , respectively). This significant reduction in chemotactic activity was not observed in cohort patients compared to matched controls.

**Conclusions:** The differences in PMN function between familial AgP and the sporadic form suggest distinct pathophysiological pathways, although leading to a similar clinical presentation. To elucidate a possible genetic predisposition testing of known variants related to PMN dysfunction is ongoing.

#### P04.007

##### Role of miRNAs in the etiology of alopecia areata: A genome-wide miRNA association analysis

*A. Tafazzoli<sup>1</sup>, A. Fostner<sup>1</sup>, A. Hofmann<sup>1</sup>, S. Redler<sup>2</sup>, L. Petukhova<sup>3</sup>, F. Basmanav<sup>1</sup>, K. Giehl<sup>4</sup>, S. Eigelshoven<sup>5</sup>, R. Kruse<sup>6</sup>, B. Blaumeiser<sup>7</sup>, M. Böhm<sup>8</sup>, N. Garcia Bartels<sup>9</sup>, G. Lutz<sup>10</sup>, H. Wolff<sup>11</sup>, U. Blume-Peytavi<sup>9</sup>, A. Christiano<sup>3</sup>, M. Nöthen<sup>1</sup>, R. Betz<sup>12</sup>;*

<sup>1</sup>Institute of Human Genetics and Department of Genomics, Bonn, Germany, <sup>2</sup>Institute of Human Genetics, Bonn, Germany, <sup>3</sup>Dermatology and Epidemiology, New York, NY, United States, <sup>4</sup>Dermatology, Munich, Germany, <sup>5</sup>Dermatology, Dusseldorf, Germany, <sup>6</sup>Dermatological Practice, Baderborn, Germany, <sup>7</sup>Medical Genetics, Antwerp, Belgium, <sup>8</sup>Dermatology, Munster, Germany, <sup>9</sup>Clinical Research Center for Hair and Skin Science, Department for Dermatology and Allergy, Berlin, Germany, <sup>10</sup>Hair & Nail, Dermatological Practice, Wesseling, Germany.

**Background:** Alopecia areata (AA) is a common hair loss disorder characterized by a sudden onset of patchy areas of hair loss, which can occur on the scalp or elsewhere on the body. Immunological and genetic association studies support the hypothesis that AA is autoimmune in nature. Recent evidence points to a significant role of miRNAs in autoimmune diseases; but the role of miRNAs in AA has not been investigated so far.

**Methods:** We performed a systematic analysis to investigate whether common variants among all known autosomal microRNAs loci contribute to AA development. Gene-based analyses were performed by VEGAS for all miRNAs listed in miRBase and their flanking sequences using the largest GWAS data set of 3,253 patients and 7,543 controls.

**Result:** As a result, 78 of the 617 investigated microRNAs showed nominally significant p-values. After correction for multiple testing, three microRNAs (miR-1237, miR-30b/d, miR-548h-2) showed significant association with AA. The most promising one was miR-30B. Target gene analyses for 3 disease associated microRNAs revealed 2,072 nominally significant predicted target genes. Gene based p-values were calculated for the predicted target genes revealing 42 of them to be significantly associated with AA after correction for multiple testing, including IL2RA, ERBB3 as genome-wide significant loci from former AA GWA-studies. By luciferase assays, we validated the site-specific regulation of IL2RA, STX17 and TNXB of miR-30B.

**Conclusion:** Our study is the first to suggest the importance of microRNAs in the pathogenesis of AA which could be of interest for development of therapies in the future.

#### P04.008

##### The effects of polymorphisms of death pathway genes and mitochondrial pathway genes in intervertebral disc degeneration

*B. Eser<sup>1</sup>, O. Eser<sup>1</sup>, E. Aslan<sup>2</sup>, H. Dolgun<sup>3</sup>;*

<sup>1</sup>Balikesir University, Balikesir, Turkey, <sup>2</sup>Afyon Kocatepe University, Afyonkarahisar, Turkey, <sup>3</sup>Ministry of Health Diskapi Yildirim Beyazit Education and Research Hospital, Ankara, Turkey.

**Introduction:** It has been proposed that apoptosis is effective on intervertebral disc degeneration. Especially death receptor pathway and mitochondrial pathway are reported to have a significant role in apoptosis mechanism. This study is the first study in which both polymorphisms and expressions of apoptotic genes in patients with intervertebral disc degeneration (IVDD) are evaluated together. The aim of our study is to determine whether polymorphisms and expressions of apoptotic genes involved in both pathways are related with grades of IVDD or not.

**Materials and Methods:** Blood and tissue samples of 100 patients diagnosed with lumbar disc degeneration were collected. Patients were divided into 2 groups according to their radiological degeneration grades as grade 2 (mild) and grade 3 and 4 (severe). Polymorphisms in Fas (rs 2234767), Bcl-2 (rs

1801018) and Bax (rs4645878) genes were determined with real-time PCR. Expressions of these genes were analyzed immunohistochemically following Histological Degeneration Scoring.

**Results:** Whereas no relationship was found among polymorphisms of Fas and Bax genes and their expressions, we have determined a relationship among GG genotype of Bcl-2 and their expressions. Additionally, we have found that ratio of Bax-positive cells is related with IVDD grades. In addition to this, we have found that radiological degeneration grades were compatible with histological degeneration scores.

**Conclusions:** GG genotype of Bcl-2 gene may influence the level of its expression and may be effective in development of IVDD. Additionally, expression of Bax gene may be related with different grades of IVDD.

#### P04.009

##### GLUT10 deficiency leads to oxidative stress and non-canonical $\alpha\beta\beta$ integrin-mediated TGF $\beta$ signalling associated with extracellular matrix disarray in arterial tortuosity syndrome skin fibroblasts

*N. Chiarelli, M. Ritelli, G. Carini, V. Cinquina, M. Colombi, N. Zoppi;*

*University of Brescia, Department of Molecular and Translational Medicine, Brescia, Italy.*

Arterial tortuosity syndrome (ATS, OMIM #208050) is a rare autosomal recessive connective tissue disorder characterized by tortuosity and elongation of the large and medium-sized arteries and a propensity towards aneurysm formation and vascular dissection. ATS is caused by mutations in SLC2A10 encoding the facilitative glucose transporter 10 (GLUT10). GLUT10 deficiency leads to the disarray of the extracellular matrix (ECM) and to the activation of the TGF $\beta$  pathway, but the pathomechanisms of ATS is still an enigma.

To discern the pathomechanisms underlying the ATS aetiology, we performed gene expression profiling and biochemical studies on skin fibroblasts. Transcriptome analyses revealed the dysregulation of several genes involved in TGF $\beta$  signalling and ECM homeostasis as well as the perturbation of specific pathways that control both the cell energy balance and the oxidative stress response. Biochemical and functional studies showed a marked increase in ROS-induced lipid peroxidation sustained by altered PPAR $\gamma$  function, which contributes to the redox imbalance and the compensatory antioxidant activity of ALDH1A1. ATS fibroblasts also showed activation of a non-canonical TGF $\beta$  signalling due to TGF $\beta$ RII disorganization, the upregulation of TGF $\beta$ RII and connective tissue growth factor, and the activation of the  $\alpha\beta\beta$  integrin transduction pathway, which involves p125FAK, p60 $\Sigma$ rc, and p38 MAPK. Stable GLUT10 expression in patients' fibroblasts normalized redox homeostasis and PPAR $\gamma$  activity, rescued canonical TGF $\beta$  signalling, and induced partial ECM re-organization. These data add insights into the dysregulated biological pathways and definition of the pathomechanisms involved in ATS. This work was supported by the Telethon Foundation (grant number GGP13167).

#### P04.010

##### Asphyxiating Thoracic Dysplasia (ATD) and Short Rib-Polydactyly syndrome type III (SRP III): clinical and molecular review of 71 families

*G. Baujat<sup>1</sup>, C. Michot<sup>1</sup>, Q. Siour<sup>1</sup>, M. Parisot<sup>2</sup>, C. Bole-Feysoz<sup>2</sup>, C. Fourrage<sup>3</sup>, P. Nitschke<sup>3</sup>,*

*H. Kayirangwa<sup>4</sup>, K. Le Quan Sang<sup>4</sup>, A. Dieux-Coeslier<sup>4</sup>, G. Mortier<sup>5</sup>, P. Simsek-Kiper<sup>6</sup>, K.*

*Chandler<sup>7</sup>, A. David<sup>8</sup>, C. Do Ngoc Thanh<sup>9</sup>, C. Francannet<sup>10</sup>, D. Gaillard<sup>11</sup>, A. Goldenberg<sup>12</sup>, A. Megarbane<sup>13</sup>, S. Sigaudo<sup>14</sup>, C. Thauvin<sup>15</sup>, T. Attié-Bitach<sup>16</sup>, S. Saunier<sup>17</sup>, Skeletal Ciliopathies Consortium, C. Huber<sup>1</sup>, V. Cormier-Daire<sup>1</sup>,  
<sup>1</sup>Service de Génétique, INSERM UMR 1163, Université Paris Descartes- Sorbonne Paris Cité, Institut Imagine, Hôpital Necker Enfants Malades (AP-HP), Paris, France, <sup>2</sup>Plateforme de Génomique, Institut IMAGINE, Paris, France, <sup>3</sup>Plateforme de Bioinformatique, Université Paris Descartes, Paris, France, <sup>4</sup>Service de Génétique Clinique, CHRU, Hôpital Jeanne de Flandre, Lille, France, <sup>5</sup>Department of Medical Genetics, Antwerp University Hospital and University of Antwerp, Edegem, Belgium, <sup>6</sup>Hacettepe University School of Medicine, Department of Pediatrics, Pediatric Genetics Unit, Ankara, Turkey, <sup>7</sup>Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Saint Mary's Hospital, Manchester, United Kingdom, <sup>8</sup>Service de Génétique Médicale, CHU Nantes, Nantes, France, <sup>9</sup>Médecine Physique et Réadaptation de l'Enfant, Hôpital Raymond Poincaré, Garches, France, <sup>10</sup>Service de Génétique Médical – CHU Estaing, Clermont-Ferrand, France, <sup>11</sup>Service de Génétique et Biologie de la Reproduction, Hôpital Maison Blanche, Reims, France, <sup>12</sup>Service de Génétique Médicale, CHU de Rouen, Rouen, France, <sup>13</sup>Unité de Génétique Médicale, Faculté de Médecine, Université Saint Joseph, Beyrouth, Lebanon, <sup>14</sup>Département de Génétique Médicale, Hôpital d'Enfant de la Timone, Marseille, France, <sup>15</sup>Génétique des Anomalies du Développement, EA4271, Université de Bourgogne, Dijon, France, <sup>16</sup>Laboratoire de Génétique Moléculaire, Institut Imagine INSERM U-1163, Hôpital Necker-Enfants Malades, Paris, France, <sup>17</sup>INSERM UMR 1163, Laboratory of Inherited Kidney Diseases, Université Paris Descartes- Sorbonne Paris Cité, Institut Imagine, Hôpital Necker Enfants Malades (AP-HP), Paris, France.*

Asphyxiating Thoracic Dysplasia (ATD) and Short Rib-Polydactyly syndrome type III (SRPIII) belong to the SRP group and are characterized by a narrow thorax, short long bones, trident acetabular roof and extraskeletal malformations. SRPIII is uniformly lethal due to pulmonary hypoplasia and other organ system abnormalities. To date, mutations in 11 genes, coding for ciliary proteins, have been reported in ATD/SRPIII. Here, we report the molecular screening of 71 individuals (53 ATD, 18 SRPIII) by targeted NGS of a customized ciliopathy gene panel, called ciliome.

The ciliome allowed us to identify the molecular basis of 70% (50/71) of ATD/SRPIII cases. Mutations in DYNC2H1 were identified in 34/50 (68%) and were not associated with kidney disease but with liver and retina involvement. Mutations in other genes, namely IFT140 (6%), WDR60 (6%), IFT144 (6%), WDR34 (4%), IFT80 (2%), WDR35 (2%) and TTC21B (2%), were associated with multiple organ damage including kidney insufficiency or liver cirrhosis illustrating the overlap with Saldino-Mainzer and Sensenbrenner syndromes. We also identified one heterozygous mutation in DYNC2H1 and IFT140 for a living ATD case, supporting a digenism. We finally identified homozygous mutations in a new ciliary gene, KIF24, in two ATD sibling fetuses with renal, hepatic and pulmonary involvement. In the 21 families with no identified molecular bases, radiological features were typical and multi organ involvement variable. We conclude that DYNC2H1 is the major gene responsible for ATD/SRP III. The exclusion of the 11 known genes in 21 families supports the involvement of other causal genes.

#### P04.011

##### A novel ATP6V0A2 frameshift mutation causing autosomal recessive cutis laxa with bleeding diathesis and defective wound healing

I. Karacan<sup>1</sup>, R. Diz Kucukkaya<sup>2</sup>, A. Tolun<sup>3</sup>, V. S. Hanger<sup>4</sup>, E. Tahir Turanlı<sup>5</sup>;

<sup>1</sup>Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey, <sup>2</sup>Department of Internal Medicine, Division of Hematology, Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey, <sup>3</sup>Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey, <sup>4</sup>Department of Molecular Biology and Genetics, Faculty of Science, Istanbul Bilim University, Istanbul, Turkey, <sup>5</sup>Dr. Orhan Ocalgiray Molecular Biology-Biotechnology and Genetics Research Center, Istanbul Technical University, Istanbul, Turkey.

**Introduction:** Autosomal recessive cutis laxa type-II (ARCL2) syndrome is a rare congenital disorder associated with growth and developmental delay as well as skeletal anomalies. Its prevalence is 1 in 12 million live births. Biallelic mutations in the responsible gene ATP6V0A2 lead to increased pH in Golgi, resulting in impaired glycosyltransferase activity and organelle trafficking. Here we present a consanguineous family with four unaffected and two affected sibs (a male and a female) with microcephaly, dysmorphic features, cleft lip/palate, vision/hearing impairment, wrinkled skin, tendency to infections, bleeding diathesis and defective wound healing.

**Materials and Methods:** SNP genotype data were used to detect regions with homozygosity shared only by the patients. Exome sequence data for the index patient were used to search for a rare, homozygous deleterious mutation in the possibly linking regions.

**Results:** Linkage analysis pointed to a large region at 12q24.21-24.32 with a multipoint LOD score of 2.58 and including 198 genes. Causative mutation search in linkage regions identified homozygous frameshift mutation p.S695RfsX12 (c.2083\_2086del) in ATP6V0A2. The truncated protein has 705 amino acids instead of the native 856.

**Conclusions:** We identified a novel disease-causing ATP6V0A2 mutation responsible for the ARCL2 in the presented family. We did not find a deleterious mutation in another gene that could possibly explain the bleeding diathesis. Furthermore, the male afflicted with ARCL2 and another brother have mental retardation for which we are searching for a causative mutation.

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#### P04.012

##### A homozygous B3GAT3 mutation causes a multisystemic cutis laxa-like syndrome, expanding the phenotype of linkeropathies

B. Guillemin<sup>1</sup>, T. Van Damme<sup>1</sup>, W. Steyaert<sup>1</sup>, D. Syx<sup>1</sup>, P. Coucke<sup>1</sup>, A. De Paepe<sup>1</sup>, S. Symoens<sup>1</sup>, S. Nampoothiri<sup>2</sup>, F. Malfait<sup>1</sup>;

<sup>1</sup>Center for Medical Genetics Ghent - Ghent University Hospital, Ghent, Belgium,

<sup>2</sup>Department of Pediatric Genetics, Amrita Institute of Medical Sciences & Research Center, Elamakkara, Cochin 682041, Kerala, India.

**INTRODUCTION:** Proteoglycans (PGs) are major components of the extracellular matrix (ECM), and fulfill an essential role as both structural and regulatory biomacromolecules. They are composed of a core protein onto which one or more glycosaminoglycan (GAG) side chains are covalently attached. Specific deficiencies in the enzymes (*XYLT1/2*, *B4GALT7*, *B3GALT6* and *B3GAT3*) involved in the biosynthesis of the tetrasaccharide linker re-

gion between the core protein and its GAGs are known as linkeropathies.

**RESULTS:** We describe the clinical features of an infant from consanguineous parents from India, in whom we identified the previously reported homozygous p.(Gly223Ser) (c.667G>A) missense mutation in *B3GAT3* using whole-exome sequencing. *B3GAT3* encodes glucuronyltransferase 1, which adds the fourth saccharide to the linker region. The boy, who expired at age 2.5 months, displayed generalized cutis laxa, osteopenia and multiple perinatal fractures, adducted thumbs, bilateral club feet and large joint contractures, severe dysmorphic features, blue sclerae and corneal clouding. Structural modeling of the substitution shows that the mutation substitutes a nonpolar Gly residue by a polar Ser residue in the catalytic pocket of the enzyme. Functional studies to further investigate the pathogenic consequences of the defect are ongoing on a cellular overexpression model of the mutant alleles. **CONCLUSIONS:** This is only the fifth reported child with a *B3GAT3* mutation. Two other missense mutations (p.(Arg277Gln) and p.(Pro140Leu)) have been reported. We highlight the extended phenotypic range of *B3GAT3* mutations and provide comparative overview of the phenotypic features of the linkeropathies associated with mutations in *XYLT1*, *XYLT2*, *B4GALT7*, *B3GALT6* and *B3GAT3*.

#### P04.013

##### Whole exome sequencing reveals a new mutation in NOD2 gene in a Mexican family with Blau syndrome

J. Toral-Lopez<sup>1</sup>, L. M. Gonzalez-Huerta<sup>2</sup>, S. Cuevas-Covarrubias<sup>3</sup>;

<sup>1</sup>Centro Médico Ecatepec, ISSEMYM, Ecatepec, Mexico, <sup>2</sup>Genetica, Hospital General de Mexico, Mexico City, Mexico, <sup>3</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.

Blau syndrome (BS; MIM 186580) and early onset sarcoidosis (EOS; MIM 609464) are familial and sporadic systemic granulomatosis diseases respectively; they are caused by heterozygous mutations in the *NOD2/CARD15* gene located on 16q12. The *NOD2* gene plays a role in nuclear NF-κappa-B activation, is associated with innate immune system and in the consequent formation of non-gaseous granulomas. BS is a genetic disorder with dominant autosomal pattern, whereas, EOS is sporadic and appears in young people. Both are clinically characterized by granulomatous arthritis, uveitis/iritis and skin rash. Twenty seven mutations on *NOD2/CARD15* gene have been associated with BS/EOS. In the present study, we analysed the *NOD2* gene from genomic in a Mexican family with BS and 100 normal controls through WES, PCR and genome DNA direct sequencing. We detected a novel mutation in the *NOD2* gene in the affected members of the family that was absent in the non-affected healthy subjects of the family and in 100 normal controls. This allowed discard a polymorphism. This is a first novel mutation observed in Mexican patients that enriches the genomic spectrum observed in BS.

#### P04.014

##### Search for variants in the *FLJ42280* genomic region and assessment of their roles in osteoporosis

N. Roca-Ayats<sup>1</sup>, M. Gerousi<sup>1</sup>, N. Martínez-Gil<sup>1</sup>, E. Czwan<sup>2</sup>, R. Urreizti<sup>1</sup>, N. Garcia-Giralt<sup>3</sup>, L. Mellibovsky<sup>3</sup>, X. Nogués<sup>3</sup>, A. Díez-Pérez<sup>3</sup>, S. Balcells<sup>1</sup>, D. Grinberg<sup>1</sup>;

<sup>1</sup>Universitat de Barcelona, IBUB, CIBERER, Barcelona, Spain, <sup>2</sup>Roche Diagnostics Deutschland GmbH, Mannheim, Germany, <sup>3</sup>URFOA, RETICEF, IMIM, Parc de Salut Mar, Barcelona, Spain.

Signals emerging from genome-wide association studies (GWAs) are frequently located in non-coding, poorly studied regions, and are likely to be in linkage disequilibrium with un-genotyped causal variants. GWAs have identified variants associated with bone mineral density (BMD) and osteoporotic fracture, some located in intronic regions of *FLJ42280*, a poorly studied gene of unknown function.

We aimed at exploring the genetic variability and functionality of this *locus*. Firstly, we resequenced the *FLJ42280* genomic region in two extreme BMD groups from the BARCOS cohort of postmenopausal women; we statistically compared the number and frequency of variants between the two groups, and we analyzed their overlap with functional elements from ENCODE. Secondly, we performed *cis*-eQTLs analyses to determine whether the GWAs SNPs, and others in strong linkage disequilibrium with them, correlated with gene expression levels.

We identified 110 variants, 18 of which were novel and 59 were low frequency variants (LFV). The number of LFV was balanced between the two extreme groups and frequency differences of all variants were below the statistical power of the design, although 9 showed trend and are currently being genotyped in the complete BARCOS cohort. One exonic variant was found and 28 variants were located in putative regulatory regions, including an interesting SNP in an active osteoblast enhancer. Functional studies of

the enhancer are currently underway. None of the SNPs showed influence on expression levels of *cis*-genes.

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#### P04.015

##### Expanding the genetic spectrum of Caffey disease

S. Symoens<sup>1</sup>, G. Mortier<sup>2</sup>, D. Wieczorek<sup>3,4</sup>, S. Unger<sup>5</sup>, A. Zankl<sup>6</sup>, T. Hospach<sup>7</sup>, P.J. Coucke<sup>1</sup>, F. Malrait<sup>1</sup>;

<sup>1</sup>Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium,

<sup>2</sup>Department of Medical Genetics, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium, <sup>3</sup>Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany, <sup>4</sup>Institut für Humangenetik, Universitätsklinikum Düsseldorf, Düsseldorf, Germany, <sup>5</sup>Medical Genetics Service, CHUV, University of Lausanne, Lausanne, Switzerland, <sup>6</sup>Academic Department of Medical Genetics, Discipline of Genetic Medicine, The University of Sydney, Sydney Children's Hospital Network, Sydney, Australia, <sup>7</sup>Pediatric Rheumatology, Klinikum Stuttgart, Olgahospital, Stuttgart, Germany.

Infantile cortical hyperostosis or Caffey disease is an autosomal dominant rare bone disorder, characterized by inflammation associated with cortical and periosteal thickening of affected bones and swelling of the surrounding soft tissues. The disease is diagnosed in young infants and is characterized by spontaneous resolution of the cortical thickening, with episodes of recurrence and reduced penetrance. The underlying genetic cause is an unique recurrent arginine-to-cysteine substitution (p.(Arg1014Cys), c.3040C>T) in the helical domain of the  $\alpha 1$ -chain of type I collagen (COL1A1).

We describe six unrelated probands with Caffey disease. Molecular analysis of COL1A1 confirmed heterozygosity for the c.3040C>T (p.(Arg1014Cys)) mutation in three probands. Biochemical collagen studies on one of these probands' dermal fibroblasts showed intracellular  $\alpha 1(I)2$ -dimers, which are at least partly secreted into the extracellular matrix. In the remaining three probands a novel c.2752C>T nucleotide change (p.(Arg918Cys)) was identified. This mutation also causes the formation of  $\alpha 1(I)2$ -dimers, which were not secreted into the extracellular matrix.

Although both arginine-to-cysteine substitutions are in relative close proximity, they affect different protein regions. While the p.(Arg1014Cys) maps to the gap region between two Major Ligand Binding Regions (MLBR2-3), the p.(Arg918Cys) is located within the cell-interacting domain MLBR2, associated with (severe-to-)lethal Osteogenesis imperfecta.

So far the mechanism driving the temporary hyperostosis and inflammatory reaction in Caffey disease is unknown. Unraveling the genetic spectrum is the first step towards our understanding of its pathophysiology. Future studies will evaluate the effect of intracellular retention of mutant collagens chains and interference with other proteins in the extracellular matrix.

#### P04.016

##### Molecular characterization of three canine models of human rare bone diseases: Caffey, van den Ende-Gupta, and Raine syndromes

M. K. Hytönen<sup>1,2,3</sup>, M. Arumilli<sup>1</sup>, A. K. Lappalainen<sup>4</sup>, M. Owczarek-Lipska<sup>5</sup>, V. Jagannathan<sup>5</sup>, S. Hundt<sup>1</sup>, E. Salmeila<sup>1</sup>, P. Venta<sup>6</sup>, E. Sarkkila<sup>4</sup>, T. Jokinen<sup>4</sup>, D. Gorgas<sup>7</sup>, J. Kere<sup>1,2,8</sup>, P. Nieminen<sup>9</sup>, C. Drögemüller<sup>5</sup>, H. Lohi<sup>1,2,3</sup>;

<sup>1</sup>Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland,

<sup>2</sup>The Folkhälsan Institute of Genetics, Helsinki, Finland, <sup>3</sup>Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, <sup>4</sup>Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland, <sup>5</sup>Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, <sup>6</sup>Department of Microbiology and Molecular Genetics, Michigan State University, Michigan, MI, United States, <sup>7</sup>Division of Clinical Radiology, Department of Clinical Veterinary Medicine, Bern, Switzerland, <sup>8</sup>Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden, <sup>9</sup>Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland.

Introduction: One to two percent of all children are born with a developmental disorder and for many of them the molecular pathogenesis remains poorly characterized. Parallel developmental disorders in other species could provide complementary models for human rare diseases by uncovering new candidate genes, improving the understanding of the molecular mechanisms and opening possibilities for therapeutic trials. Our study investigated the clinico-pathological features and genetic causes of three developmental syndromes in dogs; craniomandibular osteopathy (CMO), previously undescribed skeletal syndrome, and severe tooth wear due to hypomineralization.

Materials and Methods: We established study cohorts for each disease and characterized their clinical features. Either a genome-wide association study combined with targeted resequencing or whole genome sequencing was utilized to identify the causal variants.

Results: For the studied disorders, we identified pathogenic variants in the

canine *SLC37A2*, *SCARF2* and *FAM20C* genes, respectively. CMO is a clinical equivalent to an infantile cortical hyperostosis (Caffey disease) for which *SLC37A2* is a new candidate gene. *SLC37A2* is a poorly characterized member of a glucose-phosphate transporter family without previous disease associations. Mutations in *SCARF2* and *FAM20C* have been associated with human van den Ende-Gupta and Raine syndromes and our results demonstrate that the canine disorders show remarkable similarity to human syndromes.

Conclusions: Given the growing interest in the molecular characterization and treatment of human rare diseases, our study presents three novel physiologically relevant models for further research and therapy approaches. In addition, identification of mutations in the human orthologues provides molecular identity for the canine conditions.

#### P04.017

##### Identification and validation of novel genetic variants associating with canine hip dysplasia related traits

L. Mikkola<sup>1</sup>, S. Holopainen<sup>2</sup>, A. Lappalainen<sup>2</sup>, M. Hytönen<sup>1,3</sup>, O. Hakosalo<sup>1</sup>, H. Lohi<sup>1,4,5</sup>, A. Iivanainen<sup>1</sup>;

<sup>1</sup>Department of Veterinary Biosciences, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland, <sup>2</sup>Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland, <sup>3</sup>The Folkhälsan Institute of Genetics, Helsinki, Finland, <sup>4</sup>Haartman Institute, Helsinki, Finland, <sup>5</sup>Research Programs Unit, Helsinki, Finland.

Introduction: Canine hip dysplasia (CHD) is a common, complex, and moderately heritable disorder. The genetic background of CHD remains ambiguous despite rigorous studies. It is non-congenital, inflicts pain via secondary osteoarthritis and may lead to euthanasia on humane grounds. We aimed to identify risk loci and gene variant(s) of canine hip dysplasia and to validate our findings with replication studies.

Materials and methods: Our data consisted of German shepherd dogs. Population stratification was considered in our study. Ethylenediaminetetraacetic acid (EDTA) preserved blood samples were genotyped with a high density 173K canine single nucleotide polymorphism (SNP) array. We executed a genome-wide association study (GWAS) in two steps: 1) with a case-control data, and 2) with a larger cohort of dogs with expanded phenotypes. Validation studies were conducted with an independent cohort of dogs, using competitive allele specific PCR (KASP™) based SNP genotyping.

Results: The first GWAS revealed seven suggestive SNPs in one chromosome; the haplotype frequencies demonstrated a significant correlation with the severity of the phenotype in our validation study. The second GWAS detected one genome-wide significant and 29 suggestive SNPs in several additional chromosomes.

Conclusions: We revealed one genome-wide significant and multiple suggestive SNPs, all previously unprecedented, associating with CHD traits. Seven of these SNPs were validated indicating significant correlation with the severity of the phenotype. Additional validation studies are underway for these as well as for the remaining 30 SNPs revealed in the second GWAS. Canine health foundation and the Academy of Finland funded this study.

#### P04.018

##### Cartilage hair hypoplasia: a case with compound heterozygous two novel mutations

B. Akgun<sup>1</sup>, S. Candan<sup>2</sup>, E. Isik<sup>1</sup>, T. Atik<sup>1</sup>, A. Aykut<sup>3</sup>, A. Durmaz<sup>2</sup>, F. Ozkinay<sup>1</sup>;

<sup>1</sup>Ege University Faculty of Medicine, Department of Pediatrics, Subdivision of Pediatric Genetics, Izmir, Turkey, <sup>2</sup>Balikesir Ataturk State Hospital, Medical Genetics Department, Balikesir, Turkey, <sup>3</sup>Ege University Faculty of Medicine, Department of Medical Genetics, Izmir, Turkey.

Introduction: Cartilage hair hypoplasia (CHH) is an autosomal recessive genetic disorder characterised by short-limb dwarfism, sparse and light-colored hair, Hirschsprung's disease and immune deficiency. It is caused by mutations in the RMRP gene, which encodes the RNA component of the mitochondrial RNA-processing ribonuclease (RNase MRP). Several mutations have been identified in its promoter region or transcribed sequence.

Case report: A 6 month-old-girl was born to nonconsanguous parents at 39th week of gestation by cesarean section. Bilateral rhizomelia on the upper and lower extremities were detected at the prenatal period. She had postnatal growth retardation and chronic constipation. On physical examination, sparse and light colored hair and eyebrows, frontal bossing, antverted ears, rhizomelic dwarfism and joint laxity were observed. Neuromotor development was normal. Immunological parameters were normal. Short extremities and metaphyseal dysplasia were detected on the bone survey. We detected compound heterozygous two novel mutations [g.4976\_4989dupTACTACTTGTAA and +22T>C] on the RMRP gene by Sanger sequencing analysis.

Conclusion: In conclusion, in a case who has normal immune phenotype and

prominent skeletal findings compound heterozygous mutations in RMRP gene were reported. Clinical findings of the syndrome depend on the mutation harboring in the gene.

#### P04.019

#### Cartilage-hair hypoplasia by a new mutation 151G>C in the RMRP gene

**M. Ortiz, A. Lafuente, G. Pi, A. Zuñiga;**  
Hospital de la Ribera, Alzira (Valencia), Spain.

Cartilage-hair hypoplasia (CHH; MIM#250250) is a form of short-limbed dwarfism due to skeletal dysplasia. CHH is an autosomal recessive disorder characterized by skeletal involvement, short stature, variable features like blond fine sparse hair, and defective cellular immunity affecting T-cell-mediated responses. Patients may have a severe combined immunodeficiency requiring bone marrow transplantation or they may be asymptomatic. CHH is caused by mutations in the RMRP gene (RNA component of mitochondrial RNA-processing endoribonuclease), an untranslated gene with only 267 nucleotides that encode an RNA subunit of an RNase-MRP complex. This is the first nuclear-encoded RNA in which mutations have been found to lead to human diseases. RMRP is characterized by a very high density of SNPs and several pathogenic variants. Subsequently, molecular diagnosis remains problematic.

Proband was a 8 mo male and he is the first child of healthy, non-consanguineous parents. No family history of congenital anomalies was referred. The child was born after 38 weeks of an uneventful pregnancy. Low birth weight, short stature and forearm and hand hypoplasia, with contractures of both wrist and fingers, were the main symptoms after the birth. Karyotype was normal male and CGH array did not detect any CNV. Direct sequencing of RMRP gene detected a known pathological mutation, 195C>T (maternal origin) and a non-Previously described mutation 151G>C (paternal origin) in RMRP gene. Phylogenetic analysis indicates that may have a pathological character affecting the secondary structure of the RNase-MRP RNA. Therefore, the patient should carry two pathological mutations that confirm the diagnosis of cartilage-hair hypoplasia.

#### P04.020

#### Congenital contractual arachnodactyly: delineation of clinical criteria

**S. De Coninck<sup>1</sup>, I. Meersschaert<sup>1</sup>, S. Garcia Miñaur<sup>2</sup>, J. C. Oosterwijk<sup>3</sup>, R. Igobkwe<sup>4</sup>, M. Suri<sup>5</sup>, A. Bayat<sup>6</sup>, G. Jones<sup>7</sup>, C. I. Dali<sup>8</sup>, S. Lynch<sup>9</sup>, E. Blair<sup>10</sup>, A. Collins<sup>10</sup>, V. Laitinen<sup>11</sup>, E. Thomas<sup>12</sup>, A. Male<sup>13</sup>, I. Stolte-Dijkstra<sup>3</sup>, K. Keymolen<sup>14</sup>, L. Cheryll<sup>15</sup>, D. Yadav<sup>16</sup>, F. McKenzie<sup>17</sup>, S. Berland<sup>18</sup>, P. Willems<sup>19</sup>, A. Topa<sup>20</sup>, F. Petit<sup>21</sup>, A. Destree<sup>22</sup>, J. De Backer<sup>1</sup>, P. J. Coucke<sup>1</sup>, A. De Paepe<sup>1</sup>, S. Symoens<sup>1</sup>, B. L. Callewaert<sup>1</sup>;**

<sup>1</sup>Center for Medical Genetics, University Hospital of Ghent, Ghent University, Ghent, Belgium, Ghent, Belgium, <sup>2</sup>Clinical Genetics dpt, University Hospital la Paz, Madrid, Spain, <sup>3</sup>Dpt of genetics, University medical center Groningen, Groningen, Netherlands, <sup>4</sup>West Midlands Clinical Genetics Unit, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom, <sup>5</sup>Nottingham Clinical Genetics Service Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom, <sup>6</sup>Clinical Genetic Clinic, Kennedy Center, Copenhagen University Hospital, Copenhagen, Denmark, <sup>7</sup>Clinical Genetics Department, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom, <sup>8</sup>Department of clinical genetics, Our Lady's Children & Temple street Hospitals, Dublin, Ireland, <sup>9</sup>Oxford University Hospitals NHS Trust Churchill Hospital, Oxford, United Kingdom, <sup>10</sup>Wessex Clinical Genetics Service University Hospital Southampton NHS Foundation Trust Princess Anne Hospital, Southampton, United Kingdom, <sup>11</sup>Finlab Laboratories, Tampere, Finland, <sup>12</sup>Genetics clinic, Borough Wing Guy's Hospital Great Maze Pond, London, United Kingdom, <sup>13</sup>Clinical Genetics dpt, Great Ormond street hospital, London, United Kingdom, <sup>14</sup>Center for Medical Genetics, Brussels University Hospital, Brussels, Belgium, <sup>15</sup>Clinical genetics dpt, NHS Greater Glasgow & Clyde, Glasgow, United Kingdom, <sup>16</sup>Dpt of Pediatrics, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India, <sup>17</sup>Genetic Services of Western Australia, Subiaco, Australia, <sup>18</sup>Center for Medical Genetics and Molecular Medicine Haukeland University Hospital, Bergen, Norway, <sup>19</sup>Gendia, Antwerp, Belgium, <sup>20</sup>Dpt of Clinical Genetics, Sahlgrenska University Hospital, Göteborg, Sweden, <sup>21</sup>Dpt for clinical Genetics, CHU de Lille, Lille, France, <sup>22</sup>Center for Human Genetics, Institute for Pathology and Genetics, Gosselies, Belgium.

Congenital contractual arachnodactyly (CCA) is an autosomal dominant connective tissue disorder manifesting joint contractures, arachnodactyly, crumpled ears, and scoliosis as main features. Its rarity and substantial overlap with other conditions including Bethlem myopathy, Marfan syndrome and distal arthrogryposes, make the diagnosis challenging, though important for clinical management. CCA is caused by mutations in FBN2. We performed a comprehensive clinical and molecular assessment in a large cohort of CCA patients to delineate clinical diagnostic criteria and guide molecular analyses for FBN2.

FBN2 analysis using either Sanger Sequencing or PCR-based next-generation sequencing was performed in 122 clinically well-characterized probands. For 10 clinical characteristics, the positive and negative predictive value to find an FBN2 mutation was determined in order to establish a

weighted clinical scoring system on 20 points. Statistics were performed using SPSS 22.0.

Forty-seven probands harbored an FBN2 mutation (mutation uptake rate 39%). All but 2 mutations were located in the neonatal region (exons 22-36) with half of them altering or producing cysteines. Three patients had large intragenic deletions or frameshift mutations. Five FBN2+ patients developed cardiovascular complications. Logistic regression analysis reveals a significantly higher score in FBN2+ versus FBN2- patients ( $p<0.001$ ). Cut point analysis using a ROC curve revealed that a clinical score of 11+ yields a sensitivity of 84% and a specificity of 60% for the FBN2 result. In addition, in patients presenting with a clinical score of 11+ and at least crumpled ears and arachnodactyly, the probability of finding an FBN2 mutation is 60%. BOF15/MET-V/011; FWO:1881515N

#### P04.021

#### Rare variant in the CECR1 gene and susceptibility to autoimmune diseases?

**T. Roberta<sup>1</sup>, R. Santacroce<sup>1</sup>, A. Leccese<sup>1</sup>, A. Corrado<sup>2</sup>, M. Margaglione<sup>1</sup>;**

<sup>1</sup>Laboratory of Human Genetics, Foggia, Italy, <sup>2</sup>Clinica Reumatologica- Azienda Ospedaliero Universitaria di Foggia, Foggia, Italy.

CECR1 (cat eye syndrome chromosome region, candidate 1) encodes adenosine deaminase 2. Adenosine deaminase (ADA) catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA, an enzyme essential for the differentiation and proliferation of lymphocytes and the monocyte-macrophage system, has been used for monitoring several diseases in which immunity has been altered.

In previous studies it was observed that compound heterozygous mutations in CECR1 cause a syndrome which includes systemic vasculopathy and inflammation, showing overlap with polyarteritis nodosa and signs of compromised endothelial integrity and endothelial cellular activation. Since this syndrome shared similarities with systemic sclerosis disease (presence of antibodies and connective tissue damage), we performed a mutational analysis of the CECR1 gene on this autoimmune disease to test the hypothesis that gene may be also involved in it.

We identified one CECR1 missense mutation (p.Arg45Trp) in one patient affected by diffuse form of the disease, and it was not observed in the 200 Italian controls analysed in this study. This substitution affects a nucleotide in the dimerization domain, and in silico analysis performed with three prediction algorithms predicts for it a nonneutral role. The other 3 detected variations were synonymous and have already been described in public databases and were identified also in control samples.

The presence of only one mutation in heterozygosity cannot be responsible for the phenotype of the patient and therefore, we did not observe any significant evidence for the implication of this gene in SSc development.

#### P04.022

#### The candidate implant materials being Mg-Y-RE's alloys suppressed chondrogenesis

**B. Gogebakan, M. Izmirli, K. Gunduz, L. Atac, N. Bilgic, S. Ozarslan, O. Sahin; Mustafa Kemal University, Hatay, Turkey.**

Osteoarthritis (OA) is the most common form of joint disease and it may commonly develop knees, hips, hands, facet joints and feet. The gold standard for the diagnosis of OA is the histology of synovial cell. The synovial cell consists of from one to four layers of cells including fibroblast cell and several biomarkers for OA and chondrogenesis. For OA, the chondrocyte implantation and osteochondral autograft procedures are used as treatment of methods which are expensive and have side effects. Another method is implants being made of several materials can be used as knee implant. CHI3L1, COMP, MMP1, ADAMTS4, ADAMTS5, HIF1A, NFKB, EPAS1 genes are associated with chondrogenesis and they are well known biomarkers for OA and chondrogenesis. Therefore, we aimed to determine the invitro cytotoxicity and the level of mentioned genes expressions of synthesized Mg-Y-RE12, Mg-Y-RE13 and Mg-Y-RE24 alloys by Micro/Nano-Mechanical Characterization Laboratory of Physic department as implant materials. HEL299 which is the fibroblast cell line as model of synovial fibroblast is used. For this purpose, HEL299 was exposed to various concentrations of Mg-Y-RE12, Mg-Y-RE13 and Mg-Y-RE24 at 24, 48 and 72 hours. Cell viability was evaluated by MTT. The expression levels of genes were evaluated with qRT-PCR. To compare control and exposed HEL299 cell line, Mg-Y-RE12 suppressed COMP, ADAMTS5, HIF1A, NFKB and EPAS1 genes, Mg-Y-RE13 suppressed CHI3L1, ADAMTS5, HIF1A, NFKB, EPAS1 genes and Mg-Y-RE24 suppressed CHI3L1, ADAMTS4, ADAMTS5, MMP1, HIF1A, NFKB, EPAS1 genes. In a conclusion, Mg-Y-RE12, Mg-Y-RE13 and Mg-Y-RE24 cannot suitable for knee implant materials.

**P04.023****Cleidocranial Dysplasia - Clinical, radiological and molecular diagnosis of 5 cases**A. R. Soares<sup>1</sup>, M. E. Oliveira<sup>1</sup>, C. Melo<sup>2</sup>, G. Soares<sup>1</sup>, R. Santos<sup>1</sup>, A. M. Fortuna<sup>1</sup>;<sup>1</sup>Centro Genética Médica Dr. Jacinto Magalhães - CHPorto, Porto, Portugal, <sup>2</sup>Serviço de Pediatria - Centro Hospitalar Médio Ave, Vila Nova Famalicão, Portugal.

**Introduction:** Cleidocranial dysplasia (CCD, MIM#119600) is an autosomal dominant skeletal dysplasia characterized by hypoplasia/aplasia of the clavicles, delayed closure of the cranial sutures and multiple dental abnormalities. Other features that may be present are macrocephaly, brachycephaly and bossing, short stature and other skeletal abnormalities. The prevalence of CCD is one in 1 million. Mutations in the RUNX2 gene are responsible for 60-70% of the cases (~40% are de novo). The diagnosis is based on clinical and radiological findings and may be confirmed by RUNX2 gene mutation analysis.

**Materials and Methods:** We report 5 CCD patients (4 families), in which RUNX2 sequence analysis was performed.

**Results:** All the patients presented with typical clinical features and specific radiological findings. The RUNX2 gene molecular study revealed 2 missense mutations (in 2 families) affecting highly conserved amino acids - p.Arg190Trp and p.Lys218Asn. The later represents a novel base substitution (c.654A>C), located in the DNA-binding Runt domain. Further studies will be performed in the other 2 families.

**Conclusions:** These cases illustrate the importance of clinical clues for the diagnosis, as well as the intra and interfamilial variability of this disease. The molecular confirmation allowed a more accurate genetic counselling for patients and families, namely by the opportunity to offer prenatal diagnosis. Although CCD has an overall good prognosis, its earlier diagnosis may lead to a better management from a multidisciplinary team thereby providing an improved quality of life for patients.

**P04.024****Novel RUNX2 mutation in the runt domain in a Japanese patient with cleidocranial dysplasia**J. Machida<sup>1</sup>, H. Goto<sup>2</sup>, A. Shibata<sup>2</sup>, T. Tatematsu<sup>2</sup>, T. Nagao<sup>3</sup>, K. Shimozato<sup>2</sup>, Y. Tokita<sup>4</sup>;<sup>1</sup>Toyota Memorial Hospital, Toyota, Aichi, Japan, <sup>2</sup>Aichi-Gakuin University, Nagoya, Aichi, Japan, <sup>3</sup>Okazaki City Hospital, Okazaki, Aichi, Japan, <sup>4</sup>Institute for Developmental Research, Aichi-Human Service Center, Kasugai, Aichi, Japan.

**Introduction:** Cleidocranial dysplasia (CCD; MIM 119600) is a rare autosomal-dominant inherited disorder that causes an abnormal skeletal genesis characterized by short stature, absent or delayed closure of cranial sutures, hypoplastic or absent clavicles, and dental anomalies, such as delayed tooth eruption of permanent teeth, hypodontia, and supernumerary teeth. It has been demonstrated that hypomorphic or haploinsufficiency of the runt related transcription factor 2 (RUNX2) gene is causative for CCD. RUNX2 encodes an osteoblast-specific transcription factor, which recognizes specific DNA sequences via the runt domain, and has an important role in the differentiation of osteoblasts and the maturation of chondrocytes.

**Materials and Methods:**

Mutational analysis was performed with fragments covering the entire coding region of the RUNX2 gene. In addition, biochemical analyses were carried out to investigate the role of detected mutant RUNX2 in the CCD pathologies.

**Results and Conclusions:**

In this study we described the case of a 9 year old Japanese boy with CCD who carries a novel mutation in the RUNX2 gene (c.473c>a) that resulted in an amino acid substitution (p.A158E) in the runt domain of the gene product. Although A158E RUNX2 showed stable exogenous expression in transfected COS7 cells and a normal nuclear localization pattern, it did not show transcriptional activity in a reporter assay. This indicates that the A158E mutation in RUNX2 is causal for the CCD in our current case.

**P04.025****Mutations in COL1A1 and COL1A2 in patients with osteogenesis imperfecta**J. Díaz-Garzón<sup>1</sup>, C. Prior<sup>1</sup>, C. Gómez<sup>1</sup>, L. Pascual<sup>1</sup>, R. Núñez<sup>2</sup>, R. de Sancho<sup>1</sup>, N. Chacón<sup>1</sup>, E. Vallespín<sup>1</sup>, V. Martínez-González<sup>2</sup>, P. Gutiérrez<sup>2</sup>, J. Molano<sup>1</sup>, A. Bueno<sup>2</sup>, G. Otáñez<sup>3</sup>, S. Temtamy<sup>3</sup>, M. Aylan<sup>3</sup>, P. Lapunzina<sup>4</sup>, V. L. Ruiz-Pérez<sup>4</sup>;<sup>1</sup>Hospital La Paz, Madrid, Spain, <sup>2</sup>Hospital Universitario de Getafe, Madrid, Spain,<sup>3</sup>Human Genetics and Genome Research Division, Centre of Excellence of Human Genetics, National Research Centre, Cairo, Egypt, <sup>4</sup>Instituto Investigaciones Biomédicas, Madrid, Spain.

**Introduction:** *Osteogenesis imperfecta* (OI) is a connective tissue disorder characterized by skeletal fragility leading to recurrent fractures. It is mainly an autosomal dominant disorder with 85-90% of cases having mutations in

the type I collagen genes *COL1A1* and *COL1A2*.

The objective of this study was to identify mutations in *COL1A1* and *COL1A2* in patients with clinical suspicion of OI.

**Material and Methods:** The coding regions of *COL1A1* and *COL1A2* were screened in 47 families with OI by next-generation sequencing (NGS). Most families were Spanish, but patients from other countries were included. We confirmed the mutations by conventional Sanger sequencing. Familial analysis were performed when samples were available.

**Results:** We identified 28 different pathogenic mutations in 31 families (68%). Most common mutations were missense variants affecting glycine residues but splicing and frameshift mutations were also found. There were 12 novel mutations (*COL1A1*=6; *COL1A2*=6) and eight mutations were confirmed to be *de novo*.

**Conclusions:** NGS is a suitable method to accomplish molecular diagnosis of *osteogenesis imperfecta*. Large deletions/duplications and deep intronic mutations which were not analysed in this study could explain the low rate of mutation detection.

The large majority of pathogenic variants identified were missense mutations affecting glycine residues and thus expected to cause collagen I triple helix disturbance.

**P04.026****Mutation in filamin B co-segregating with congenital talipes equinovarus in a chinese family**

H. Yang, Z. Zheng, H. Cai, Z. Wang, Q. Fu;

Shanghai Children's Medical Center, Shanghai Jiao Tong University school of Medicine, Shanghai, China.

**Introduction:** Congenital talipes equinovarus, also known as clubfoot, is one of the most common musculoskeletal disorders, and its etiopathogenesis is not clearly known. Genetic factors are known as contributors to its pathogenesis. Up to date, a large number of genes were involved in this process, such as PITX1, TBX4 and RBM10. Our aim was to determine the candidate disease-causing mutations in Chinese patients with isolated clubfoot.

**Materials and Methods:** Genomic DNA was extracted from peripheral blood samples of isolated clubfoot pedigree and 53 sporadic patients. Whole exome sequencing (WES) and Sanger sequencing were performed for identifying disease-causing mutations and validation, respectively.

**Results:** A putative pathogenic mutation c.4717G>T (p.D1573Y) in filamin B (FLNB) gene was successfully identified by WES and was shown to co-segregate with the family anomaly in a three-generation family with six isolated clubfoot affected individuals. In addition, two novel missense mutations in FLNB [c.1897A>G(p.M633V) and c.2195A>G(p.Y732C)] were further identified from 53 sporadic patients with isolated clubfoot.

**Conclusions:** Our results indicated that FLNB mutations were associated with isolated clubfoot, and provide a concrete evidence for involvement of FLNB in pathogenesis of isolated clubfoot and expand the clinical spectrum of FLNB mutations.

**P04.027****In vitro characterization of the skeletal stem cell niche from calvarial sutures of nonsyndromic craniosynostosis**

L. Di Pietro, M. Barba, L. Massimi, P. Frassanito, M. Caldarelli, G. Tamburini, W. Lattanzi;

Università Cattolica del Sacro Cuore, ROMA, Italy.

Nonsyndromic craniosynostosis (NSC) is a congenital defect due to the premature fusion of skull sutures. These are mesenchymal gaps that bridge the gaps among flat bones of the skull, and serve as key growth centers coordinating skull-brain concerted development. The suture mesenchyme houses a craniofacial-specific skeletal stem cell niche, recently characterized. Recent evidence suggests that NSC is due to a stem cell abnormality at this site. Our aim is to characterize the stem cell population isolated from open and fused sutures of NSC patients, focusing on the expression of lineage-specific markers.

Calvarial stem cells (CMSC) were isolated from NSC biospecimens and clonally diluted. The expression of bone marrow stem cell markers (THY1, a skeletal stemness-marker; TIE2, tyrosine kinase recognized as haemopoietic/angiogenic marker; ENPEP, glutamyl aminopeptidase involved in osteogenic differentiation of bone marrow stem cells; and ITGAV, an integrin playing a key role in bone marrow niche environment) was analyzed both in suture tissues and matched cell clones, with and without osteogenic induction.

CMSCs clones homogeneously displayed a THY1+/TIE2-/ENPEP- immunophenotype, indicating that explant cultures allow a significant selection of cell populations for both sutures and synostoses. Cells expressed increased levels of THY1 and ITGAV, while decreased levels of ENPEP and TIE2, com-



pared with matched tissue samples. The expression of THY1, ENPEP and TIE2 decreased after the osteogenic induction.

THY1+ represent reasonably the stem cell population inside the calvarial niche, hence a potential target of therapies aimed at reducing the increased osteogenic rate at the site of premature suture fusion.

#### P04.028

#### Bardet Biedl Syndrome-gene 9 (BBS9) and primary cilium signaling in the aberrant osteogenic process underlying non-syndromic craniosynostosis

*M. Barba, L. Di Pietro, L. Massimi, M. Geloso, F. Michetti, C. Di Rocco, G. Tamburrini, C. Berardini, W. Lattanzi;*

*Università Cattolica del Sacro Cuore, ROMA, Italy.*

Nonsyndromic craniosynostosis (NSC) is a congenital malformation due to the premature ossification of calvarial sutures, with an unclear multifactorial etiopathogenesis. The Bardet Biedl Syndrome-associated gene 9 (BBS9), encoding a structural component of the primary cilium, has been associated to the NSC phenotype in a GWAS. The role of BBS9 in the osteogenic commitment and differentiation of somatic stem cells inside skull bone and sutures is unknown.

The aim of this study was to investigate how BBS9 modulation affect ciliogenesis and osteogenesis, in cells isolated from fused (p-CMSC) and unfused (n-CMSC) sutures of NSC patients.

Cells were cultured either in standard growth or in osteoinductive conditions; BBS9 was silenced using siRNA, and its expression analyzed through qPCR. The number of primary cilium-expressing cells, and the activity of the cilium-related Hedgehog pathway were analyzed comparatively in all cells. BBS9 expression was higher in p-CMSC compared with n-CMSC, and underwent a further increase upon osteogenic induction. The number of ciliated cells was lower in p-CMSC compared with n-CMSC. Following BBS9 silencing, the percentage of ciliated cells raised significantly in p-CMSC, equalizing to the percentage observed in n-CMSC. Furthermore, the expression of Hedgehog pathway-related genes (SMO, GLI1, GLI3, IGF1) in p-CMSC was significantly dysregulated, compared with n-CMSC, and reverted to normal upon BBS9 silencing.

Taken together, our data point towards a functional role of BBS9 in the osteogenic commitment of suture-derived cells, highlighting a key role of the primary cilium in the abnormal osteogenic process underlying NSC pathogenesis.

#### P04.029

#### Differential expression of candidate genes for skull globularization in nonsyndromic craniosinostosis patients: a common molecular network in skull shape evolution, craniofacial dysmorphology and cognitive disabilities?

*W. Lattanzi<sup>1</sup>, L. Di Pietro<sup>1</sup>, M. Barba<sup>1</sup>, C. Boeckx<sup>2</sup>, L. Massimi<sup>1</sup>, D. Chieffo<sup>1</sup>, G. Tamburrini<sup>1</sup>, A. Benitez-Burraco<sup>3</sup>;*

*<sup>1</sup>Università Cattolica del Sacro Cuore, Rome, Italy, <sup>2</sup>ICREA/Universitat de Barcelona, Barcelona, Spain, <sup>3</sup>Universidad de Huelva, Huelva, Spain.*

Nonsyndromic craniosynostosis (NCS) is a congenital malformation, with unclear etiopathogenesis, due to premature suture fusion, which alters skull-brain landmarks. Cognitive impairment, typically involving the language domain, has been reproducibly found in NCS patients.

In a paleoneurobiology perspective, the skull of anatomically-modern humans (AMHs) has evolved to a more globular shape, compared to Neandertals, while improving cognition and linguistic abilities. Recent studies from our group pointed out that critical genes have been selected in AMHs and should be crucial for skull globularization, while playing a putative role in neurogenesis, neo-cortex and subcortical patterning, neuronal interconnection, and synaptic plasticity.

Hence we aimed to investigate the role of candidate genes for skull globularity in the premature ossification of calvarial sutures occurring in NCS, chosen as a model disease.

To this aim, these selected genes were comparatively analyzed through qPCR in sutures of NCS patients versus control samples from unaffected individuals. Differential expression of DLX5, ROBO1, SLIT2, NCAM1, TGFB2, DCN, RUNX2, and SFRP2 was found. The expression levels of these genes was also significantly modulated in cells induced towards osteogenic differentiation *in vitro*.

Taken together our data seem to suggest that the molecular signaling acting at the site of premature suture fusion in NCS partially overlaps with the gene regulatory network that shaped the skull and the brain during human evolution and that improved our cognitive abilities. We claim that this overlapping may be informative of the common molecular machinery regulating the skull-brain concerted development and evolution.

#### P04.030

#### Mutations in the E subunit of the vacuolar ATPase complex cause a novel type of autosomal recessive cutis laxa

*T. Van Damme<sup>1</sup>, A. Kariminejad<sup>2</sup>, M. Mohamed<sup>3</sup>, B. Guillemin<sup>4</sup>, T. Gardeitchik<sup>5</sup>, W. Steyaert<sup>1</sup>, S. Ghaderi-Sohi<sup>2</sup>, E. Morava<sup>4</sup>, D. Syx<sup>1</sup>, D. Lefever<sup>3</sup>, P. Coucke<sup>1</sup>, S. van Kraaij<sup>3</sup>, D. Dallayaux<sup>3</sup>, R. De Rycke<sup>5</sup>, A. De Paepe<sup>1</sup>, S. Symoens<sup>1</sup>, R. Wevers<sup>3</sup>, F. Malfait<sup>1</sup>, B. Callewaert<sup>1</sup>,*

*<sup>1</sup>Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium,*

*<sup>2</sup>Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran, Islamic Republic of,*

*<sup>3</sup>Departments of Laboratory medicine, Pediatrics and Genetics, Radboudumc, Nijmegen, Netherlands, <sup>4</sup>Hayward Genetics Center, New Orleans, LA, United States, <sup>5</sup>Department of Biomedical Molecular Biology, Inflammation Research Center, Ghent University, Ghent, Belgium.*

**Introduction:** Acidification of vesicular compartments is crucial for the function of intracellular trafficking pathways. Defects in this process due to mutations in genes encoding subunits of the vacuolar-type H<sup>+</sup>-ATPase (V-ATPase) pump cause very different Mendelian disorders. We now identified a homozygous mutation in another subunit of the V-ATPase in a consanguineous Iranian pedigree with a severe cutis laxa phenotype.

**Results:** Using whole-exome sequencing, we detected a homozygous p.(Leu128Pro) (c.383T>C) missense mutation in *ATP6V1E1*, encoding the E subunit of cytoplasmic V1 part of the V-ATPase complex, in a patient with severe and generalized cutis laxa, arthrogryposis, congenital hip dysplasia, and severe aortic root dilatation and biventricular hypertrophy. Located in the head of this subunit, Leu128 engages a hydrophobic reaction with the opposing B subunit that is predicted to be abolished by the Pro substitution. Proband fibroblasts displayed abnormal swelling and fragmentation of the Golgi apparatus on transmission electron microscopy (TEM) and severely delayed retrograde translocation of Golgi membranes to the endoplasmic reticulum after Brefeldin A treatment, suggesting disturbed vesicular intracellular trafficking. TEM analysis of the proband's dermis showed severe changes in the amount, structure and organization of elastic and collagen fibers, linking this disorder to other types of cutis laxa. Biochemical studies investigating the effect on elastin and collagen are currently ongoing.

**Conclusions:** This study describes a novel type of cutis laxa caused by mutations in the E subunit of the V-ATPase complex that affects intracellular trafficking and secretion of major extracellular matrix constituents.

#### P04.031

#### A New Face of Inherited Recessive Cutis Laxa: ATP6V0A2 gene mutations associated with metabolic defects

*G. Ogur<sup>1</sup>, F. Malfait<sup>2</sup>, U. Abur<sup>3</sup>, A. Yilmaz<sup>4</sup>, H. Mutlu Albayrak<sup>4</sup>, E. Altundag<sup>4</sup>, C. Aygün<sup>5</sup>,*

*<sup>1</sup>Ondokuz Mayıs University Medical Faculty, Genetics, Samsun, Turkey, <sup>2</sup>Ghent University, Genetics, Ghent, Belgium, <sup>3</sup>Ondokuz Mayıs University Medical Faculty, Genetics, Samsun, Turkey, <sup>4</sup>Ondokuz Mayıs University Medical Faculty, Genetics, Samsun, Turkey, <sup>5</sup>Ondokuz Mayıs University Medical Faculty, Neonatology, Samsun, Turkey.*

**Introduction.** Cutis laxa (CL) is a rare disorder of elastic fibers and is characterized by lax skin, skeletal and developmental anomalies. Several forms have been described. ARCL2A (OMIM 219200) presents lax skin with persistent wide fontanel, intrauterine growth retardation, hip dislocation, scoliosis, and inguinal hernia. A subgroup of patients with ATP6V0A2 gene mutations have also metabolic problems mostly N- and O-glycan biosynthesis defect.

Here we present two cases of ARCL2A both with homozygous ATP6V0A2 gene mutations, in one of whom, ethylmalonic aciduria was also observed. Case 1. A 6 year-old male patient from consanguineous parents was referred because of lax skin and a previous sib affected with CL. He showed a large fontanel and lax skin. Mutation analysis revealed homozygous ATP6V0A2 gene mutations, (c.187C>T, p.R63X). No mutations were identified in the FBLN5 gene.

Case 2 was a 17 day-old baby with facial dysmorphism, congenital hip dysplasia, a wide anterior fontanel, lax skin and pectus excavatum. Mutation analysis revealed homozygous nonsense ATP6V0A2 gene mutation, (c.187C>T, p.R63X). During follow up, urine organic acid screening tested because of developmental delay, revealed high levels of ethylmalonic aciduria. A repeated analysis supported the same result.

**DISCUSSION:** To best of our knowledge no previous association of cutis laxa syndrome with ethylmalonic aciduria has been reported. Increased ethylmalonic acid in urine is observed in a number of inborn errors of metabolism, as well as in people with the SCAD coding region polymorphism. It is of interest that SCAD and ARCL2A are on the same DNA loci, 12q24.31.

**P04.032****Multiple trichoepitheliomas and vulvar cysts: Extending the cutaneous phenotypic spectrum in CYLD mutation carriers**

A. Dubois<sup>1</sup>, V. Wilson<sup>1</sup>, V. Bajaj<sup>2</sup>, Á. Alonso Sánchez<sup>3</sup>, A. Husain<sup>2</sup>, N. Rajan<sup>1</sup>;  
<sup>1</sup>Institute of Genetic Medicine, Newcastle upon Tyne, United Kingdom, <sup>2</sup>Department of Dermatology, University Hospital of North Durham, Durham, United Kingdom, <sup>3</sup>Department of Pathology, RVI, Newcastle upon Tyne, United Kingdom.

Patients with germline mutations in CYLD are recognised to develop a range of patterns of presentation, including familial cylindromatosis (FC), Brooke-Spiegler Syndrome (BSS) and multiple familial trichoepitheliomas (MFT). Here we present a case of a 29-year-old female patient with multiple facial and genital papules, a pattern not previously associated with CYLD mutation.

The patient began to develop multiple, asymptomatic, skin coloured papules around the nose and upper lip from the age of 16. There was no family history of skin tumours. White, firm papules began to develop within the labia majora from the age of 25, and numbered more than 20. A biopsy of the facial lesions showed trichoepithelioma. The labial lesions demonstrated histological features consistent with epidermoid cysts. Sanger sequencing of the coding exons of CYLD revealed a novel heterozygous base duplication in exon 8, c.966dupG, resulting in a frameshift mutation.

Pubic tumours are a feature recognised in CYLD mutation carriers, and one that challenges the paradigm of ultraviolet radiation induced tumour induction. We undertook local review of records from four further patients carrying a CYLD mutation (c.2460delC) who had undergone excision of genital lesions, and none were found to have epidermoid cysts. A search of the literature did not reveal this pattern of presentation in other patients with CYLD mutations.

MFT may be genetically heterogeneous, with only 44% of patients demonstrating germline mutations in CYLD. Our finding of MFT and labial cysts may represent an infrequent, novel pattern of presentation associated with CYLD mutation.

**P04.033****Complete and partial XYLT1 deletion in a patient with neonatal short limb skeletal dysplasia**

S. van Koningsbruggen, H. Knoester, R. Bakx, O. Mook, L. knegt, J. Cobben; Academic Medical Centre, Amsterdam, Netherlands.

A boy was born with a neonatal short limb skeletal dysplasia and respiratory problems. Array CGH identified a large deletion on chromosome 16p13, which was also present in his healthy mother. However, detailed analysis of the deleted region pointed at candidate gene XYLT1, a gene reported to be associated with skeletal development. Sequence analysis was performed on the remaining allele and identified a small intragenic deletion, originating from his father. Subsequent RNA studies using NGS technology revealed several out-of-frame transcripts.

XYLT1 mutations have recently been reported as causative in recessive Desbuquois skeletal dysplasia (DBSD), but the skeletal features in our patient do not fit this diagnosis. It is possible that the phenotype of XYLT1 mutations extends to more aspecific types of short limb skeletal dysplasias and not to DBSD alone.

**P04.034****Combination of palmoplantar keratoderma and hair shaft anomalies: a warning signal of severe cardiac disorder? A systematic review on desmosomal diseases**

L. Polivka, S. Hadj-Rabia, C. Bodemer; Hôpital Necker, Paris, France.

Introduction. Inherited desmosomal diseases are characterized by skin and/or cardiac features. The dermatological features might be a clue in the determination of the underlying life-threatening cardiac disease. We propose a clinical algorithm in order to identify the patients at high risk of cardiac involvement and to orientate gene sequencing.

Methods. Systematic review of published articles. The inclusion/exclusion criteria were: 1) at least one identified mutation in the following desmosomal genes: JUP, PKP1, PKP2, DSP, DSG1 to 4, DSC1-3 and CDSN; 2) Description of the dermatological phenotype.

Results. 78 articles fulfilling the criteria were published. They correspond to 458 patients. Palmoplantar keratoderma (PPK), hair shaft anomalies (HSA), and skin fragility were the major features. Isolated PPK or isolated HSA are associated to a desmosomal disease limited to skin. The combination of PPK and HSA was recorded in 161 patients, and this association is at high risk of cardiac disease (129/161 patients, 80.1%). Skin features appeared as the initial clinical manifestations in the majority of cases. However, they had led to cardiac monitoring in only 2.3% of those patients. We delineated three major phenotypes: - the PPK-HSA-non fragile skin subtype (77%), always

associated to cardiac involvement; - the PPK-HSA-skin fragility-normal cardiac function subtype (19.9%) frequently associated Plakophilin1 anomalies; - the PPK-HSA-skin fragility-cardiac involvement subtype (3.1%) always due to DSP mutations. Three mutation hotspots in DSP and JUP account for 90.8% of the patients with cardiac involvement.

Conclusion. The combination of PPK and HSA justify long-term cardiac monitoring.

**P04.035****Dyschromatosis universalis hereditaria caused by a recessive mutation in ENPP1 gene**

M. Chourabi<sup>1,2</sup>, J. N. Foo<sup>3</sup>, D. H'mida-Ben Brahim<sup>2</sup>, L. Boussofara<sup>4</sup>, C. Bonnard<sup>1</sup>, J. E. Common<sup>5</sup>, B. Srika<sup>6</sup>, N. Ghariani<sup>7</sup>, A. Aounallah<sup>4</sup>, O. Mamaï<sup>7</sup>, R. Nouira<sup>4</sup>, B. Lane<sup>5</sup>, J. Liu<sup>3,7</sup>, A. Saad<sup>2</sup>, M. Denguezli<sup>4</sup>, B. Reversade<sup>1</sup>;

<sup>1</sup>Laboratory of Human Genetics and Embryology, Institute of Medical Biology, A\*STAR, Singapore, Singapore, <sup>2</sup>Laboratory of Human Cytogenetic, Molecular Genetics and Reproductive Biology, Farhat Hached University Hospital, Sousse, Tunisia, <sup>3</sup>Human Genetics, Genome Institute of Singapore, A\*STAR, Singapore, Singapore, <sup>4</sup>Department of Dermatology and Venereology, Farhat Hached University Hospital, Sousse, Tunisia,

<sup>5</sup>Laboratory of Epithelial Biology, Institute of Medical Biology, A\*STAR, Singapore, Singapore, <sup>6</sup>Department of Pathological Anatomy and Cytology, Farhat Hached University Hospital, Sousse, Tunisia, <sup>7</sup>Saw Swee Hock School of Public Health, National University Health Systems, National University of Singapore, Singapore, Singapore.

Dyschromatosis Universalis Hereditaria (DUH) is a rare genodermatosis characterized by asymptomatic hyper- and hypo-pigmented macules over the body. These irregular lesions appear in infancy or early childhood. DUH is subtyped into different forms. The genetic etiology of autosomal recessive DUH remains unknown.

In this study we investigated 8 patients belonging to 3 related Tunisian families with autosomal recessive DUH presents with punctuate palmoplantar keratosis (PPK). To find the causative gene, we performed homozygosity mapping followed by trio whole-exome sequencing.

In the mapped candidate locus on chromosome 6q, a unique single nucleotide variant in the ENPP1 gene was identified. Sanger sequencing confirmed the segregation of this variant (c.358T>C; p.Cys120Arg) with the disease in the 3 families. ENPP1 encodes the ectonucleotide pyrophosphatase/phosphodiesterase 1, a cell surface enzyme that catalyses the hydrolysis of ATP to AMP and generates extracellular inorganic pyrophosphate. ENPP1 has been shown to play a role in skin pigmentation as the underlying cause for dominant Cole disease. Whereas Cole Disease mutations are heterozygous and affect conserved cysteine residues in the SMB2 domain of ENPP1, this novel Cys120 missense mutation is located in the SMB1 domain and must be in the homozygous state to cause DUH.

We suggest that these two genodermatoses represent a clinical spectrum of dyschromatosis, where this newly identified DUH stands for a recessive, more severe and extended form of Cole Disease.

**P04.036****Dystrophic Epidermolysis Bullosa in two sisters**

S. Garcia<sup>1</sup>, A. M. Poyatos<sup>2</sup>, A. Rubio<sup>1</sup>, M. Martinez<sup>3</sup>, S. Padrinaci<sup>3</sup>, T. De Haro<sup>1</sup>;

<sup>1</sup>Hospital San Cecilio, Granada, Spain, <sup>2</sup>AGS Sur- Hospital de Santa Ana, Motril, Spain,

<sup>3</sup>Hospital Virgen de las Nieves, Granada, Spain.

Severe generalized recessive dystrophic epidermolysis bullosa (RDEB-sev gen) is the most severe subtype of dystrophic epidermolysis bullosa. Is a severe skin disorder beginning at birth characterized by extremely fragile skin: blisters and skin erosions occur with minimal trauma and this result in mutilating scarring and contractures of the hands, feet, and joints. Affected individuals have an increased risk of developing aggressive squamous cell carcinoma.

The reported prevalence varies between populations, from 1/1,250,000 inhabitants in Scotland to 1/2,381,000 inhabitants in the United States. In Spain the estimated prevalence is 0.8/100000.

The disease is caused by null mutations within the type VII collagen gene (COL7A1) that usually lead to a lack of functional collagen VII, the main constituent of anchoring fibrils that anchor the basement membrane to the dermis.

We present the case of two sisters of 28 and 32 years, they have clinical diagnosis of the disease and family history of parental consanguinity. The older sister has a healthy baby born by caesarean section. Gene sequencing of COL7A1 gene shows the homozygous mutation c.469insA (c.497dupA). The RDEB-sev gen comprises two types based on inheritance pattern: Dominant DEB and Recessive DEB (more severe). Molecular characterization of pathogenic variants is the only accurate method to determine mode of inheritance and recurrence risk; phenotype severity and EM/IF findings alone are not sufficient. The offspring of an individual with RDEB are obligate heterozygotes (carriers) for a pathogenic variant in COL7A1.

## P04.037

### Zebrafish modeling of the $\beta$ 4GalT7-deficient type of Ehlers-Danlos syndrome

S. Delbaere, T. Van Damme, A. Willaert, S. Symoens, P. Coucke, F. Malfait;  
Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium.

**Introduction:** Proteoglycans are found on the surface of animal cells and in the extracellular matrix. They consist of glycosaminoglycan (GAG) side chains attached to a core protein through a linker region. Biallelic mutations in *B4GALT7*, the gene encoding galactosyltransferase I ( $\beta$ 4GalT7) which is an essential enzyme for the biosynthesis of the tetrasaccharide linker region, are the cause of the rare progeroid variant of the Ehlers-Danlos syndrome (EDS). This disorder, which lacks a relevant *in vivo* animal model, is mainly characterized by short stature, hypotonia, a progeroid facial appearance and skeletal abnormalities.

**Methods:** We developed and characterized a knockdown (KD) zebrafish model for the progeroid type of EDS by using morpholino injections targeted against *b4galt7*.

**Results:** Morphant embryos showed morphological abnormalities such as a small, round head, withdrawn jaw, more front-facing eyes and mild developmental delay compared to wild-type and negative control embryos. The total amount of sulfated GAGs was severely reduced in morphant embryos and whole-mount immunohistochemistry showed that heparan and chondroitin sulfate proteoglycans were severely diminished in the heads of *b4galt7* KD embryos. In addition, alcian blue staining demonstrated that cartilage structure in the head of morphant embryos are absent or strongly misshapen and immunohistochemical staining revealed a disturbed filamentous actine pattern in their head and tail.

**Conclusion:** A *b4galt7* KD zebrafish model has been developed which seems to be specific as it mimics partly the human phenotype of patients suffering from progeroid EDS. Currently, a *b4galt7* knockout zebrafish model is being created using the CRISPR/Cas technology.

## P04.038

### Homozygous missense COL5A2 mutation associated with Ehlers-Danlos Syndrome

B. Geckinli<sup>1</sup>, K. Delil<sup>1</sup>, M. A. Soylemez<sup>1</sup>, A. Turkyilmaz<sup>1</sup>, H. Simsek<sup>1</sup>, Z. Gormez<sup>2</sup>, H. Demirci<sup>2</sup>, B. Yuceturk<sup>2</sup>, A. I. Gune<sup>1</sup>, A. Arman<sup>1</sup>;

<sup>1</sup>Medical Genetics Department, Marmara University Medical School, Istanbul, Turkey, <sup>2</sup>Advanced Genomics and Bioinformatics Research Center, The Scientific and Technological Research Council of Turkey (TUBITAK), Kocaeli, Turkey.

Classic Ehlers-Danlos syndrome is a rare autosomal dominant connective tissue disorder, characterized by skin hyperextensibility, articular hypermobility, and tissue fragility. It is caused by collagen alpha-1(V) gene (COL5A1) or the collagen alpha-2(V) gene (COL5A2) mutations, causing deficiency of type V collagen.

We report a 16 year-old girl who was born with uncomplicated pregnancy at term to unrelated parents. She was referred to our clinic because of dysmorphic features, hand deformities, operated congenital hip dislocation, thrombocytopenia, splenomegaly, and previously followed-up ventricular septal defect. On physical examination, her weight was 70 kg (90-97P), height 161 cm (25-50P) and occipitofrontal circumference 52 cm (<3P). She had mild intellectual impairment. Her clinical features were short and webbed neck, a low posterior hairline, myopia, high palate, small and deformed hands, abnormal palmar creases, pes planus, hallux valgus and striae rubra on lumbar skin. X-ray showed, loss of cervical lordosis and mild scoliosis. Homozygosity mapping with whole exome sequencing identified a homozygous missense mutation c.3794A>G (p.Asp1265Gly) in COL5A2 exon 51, affecting C-terminal propeptide domain. Her parents were heterozygous carriers for the same substitution. Previously, this homozygous mutation has not been reported in Ehlers-Danlos syndrome.

## P04.039

### Adding to the knowledge of the phenotype of musculocontractural Ehlers-Danlos syndrome caused by mutations in the DSE gene

S. Markholt<sup>1</sup>, U. B. Jensen<sup>1</sup>, C. K. Lautrup<sup>2</sup>;  
<sup>1</sup>Aarhus University Hospital, Aarhus N, Denmark, <sup>2</sup>Aalborg University Hospital, Aalborg, Denmark.

**Introduction:** The diagnosis musculocontractural Ehlers-Danlos syndrome (mEDS) has recently been discovered. Most of the patients have been diagnosed with mEDS type I and mutations in the *CHST14* gene. Only 3 patients have been published with mEDS type II caused by mutations in the *DSE* gene and the phenotype is less well-described. We present detailed phenotype of an 18 years old male patient with mEDS type II.

**Clinical findings:** The patient was the second child of consanguineous parents of Turkish descent. In infancy he presented with hypertelorism, large anterior fontanel, flattened occiput, low hair line and wide neck, bilateral adducted thumbs and arachnodactyly. He had umbilical hernia, diastasis recti, constipation, bilateral hydronephrosis, as well as cryptorchidism. He had delayed psychomotor development. MRI of cerebrum showed a cortical heterotopia of 5-12 mm, interpreted as neuronal migration defect. He had myopia, strabismus and mild unilateral perceptive hearing loss. He was diagnosed with scoliosis, joint hypermobility, repeated shoulder luxations, and had surgery for pes cavus. The skin was hyperextensible with delayed wound healing, easy bruising and development of large hematomas.

**Results:** No mutations were found in the *CHST14* gene. Sequencing of the *DSE* gene revealed homozygosity for a stop mutation c.960T>A (p.Tyr320\*). **Conclusion:** This is the first time cryptorchidism, hydronephrosis, joint dislocations, myopia and hearing impairment are described as part of mEDS type II. The phenotype of mEDS type I and type II seem very similar, although the impression is that the phenotype in mEDS type II is moderately milder.

## P04.040

### A girl with FKBP14-related Ehlers-Danlos syndrome and secondary mitochondrial dysfunction in muscle biopsy - a case report

J. Pilch<sup>1</sup>, C. Fauth<sup>2</sup>, M. Kajor<sup>3</sup>, E. Karczmarewicz<sup>4</sup>, M. Witsch-Baumgartner<sup>2</sup>, Pieikutowska-Abramczuk<sup>5</sup>, E. Jamroz<sup>1</sup>;

<sup>1</sup>Department of Paediatrics and Developmental Age Neurology, Medical University of Silesia, Katowice, Poland, <sup>2</sup>Division of Human Genetics, Innsbruck Medical University, Innsbruck, Austria, <sup>3</sup>Department of Histopathology, Medical University of Silesia, Katowice, Poland, <sup>4</sup>Department of Biochemistry, Radioimmunoassay, and Experimental Medicine, The Children's Memorial Health Institute, Warsaw, Poland, <sup>5</sup>Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland.

FKBP14-related Ehlers-Danlos syndrome (EDS) is a recently identified inherited connective tissue disorder characterized by severe muscular hypotonia at birth, progressive kyphoscoliosis, joint hypermobility, hyperelastic skin, myopathy, hearing loss and vascular complications. Due to severe muscle involvement patients frequently undergo muscle biopsy which sometimes may yield unusual findings.

We present an 8-year-old girl with FKBP14-related EDS and COX negative fibers in muscle biopsy. She was born prematurely with microcephaly, hip dysplasia, valgus deformity of feet, severe muscular hypotonia and muscle atrophy. During the first months of life excessive sweating and hyperlactacidemia was noted. Motor milestones were delayed. Acyl carnitine profile in serum and transferrin electrophoresis were normal. EMG showed a myopathic pattern which prompted us to muscle biopsy. Histopathology and EM showed no structural abnormalities. However, COX staining was negative and activity of respiratory chain complexes II+III was reduced. Thus, a mitochondrial disorder was suspected and selected mitochondrial genes (SURF1, SCO2 and MTATP6) were screened but no mutation was found. During infancy motor function improved, but joint hypermobility was noted and she developed hearing impairment, easy bruising, chest deformity and scoliosis starting at the age of 7 years. Based on these findings FKBP14-deficient EDS was suspected and confirmed by sequence analysis which revealed the recurrent homozygous c.362dupC mutation in FKBP14 gene. In conclusion, muscle biopsies in FKBP14-related EDS may sometimes yield unusual findings such as mitochondrial dysfunction which probably is a secondary finding. However, the exact pathophysiology of FKBP14-related EDS and its impact on muscle function still remain to be elucidated.

## P04.041

### Significance and correlation of ultrastructural and molecular genetic approaches in the diagnostics for Ehlers-Danlos syndrome (EDS) patients with the classic and vascular type including the potential of next-generation sequencing (NGS)

K. Mayer<sup>1</sup>, I. Hausser<sup>2</sup>, R. Brumm<sup>1</sup>, S. Doecken<sup>1</sup>, H. Klein<sup>1</sup>;

<sup>1</sup>Center for Human Genetics and Laboratory Diagnostics (AHC), Martinsried, Germany, <sup>2</sup>Institute of Pathology Heidelberg (IPH), Heidelberg, Germany.

EDS represents a clinically and genetically heterogeneous group of rare connective tissue disorders (CTDs) with challenges in establishing a precise testing strategy to define an accurate diagnosis. Six main EDS subtypes can be assigned clinically and substantiated by investigations like ultrastructural analysis of a skin biopsy and genetic analysis of collagen or collagen modifying genes.

Matching of pseudonymized data sets consisting of morphological investigations from 1511 patients achieved between 1984 and 2015 and genetic analyses of 1187 patient samples performed between 2002 and 2015 resulted in an intersection of 157 cases. 45 specimen were morphologically

compatible with the classic type, 45 with the vascular type, 16 with the hypermobile type, 26 with a CTD without specification, 25 showed no pathological morphology.

With respect to the morphological classification, in 10/45 cases with the classic and 19/45 cases with the vascular assignment the diagnosis was confirmed by a mutation identified with Sanger sequencing in COL5A1/2 or COL3A1, respectively. To uncover the genetic basis in mutation negative patients with a clear morphological assignment to the classic (35) or vascular (26) group, we performed NGS applying a panel of 41 genes known to be mutated in different EDS subtypes or related CTDs like cutis laxa, Loes Dietz syndrome, Marfan syndrome and TAAD. In addition, whole-exome sequencing was undertaken to identify mutations not previously associated with EDS. In preliminary results of 19 reanalyzed patient samples gene panel analysis determined a molecular diagnosis in one case with classic morphology and in three cases with vascular morphology.

#### P04.042

##### Contribution of NGS in the Ehlers-Danlos syndromes diagnosis and characterization

A. Legrand<sup>1,2,3</sup>, V. Boccio<sup>4</sup>, A. Talbi<sup>1</sup>, X. Jeunemaitre<sup>1,2,3</sup>, J. M. Albuison<sup>1,4,5</sup>,

<sup>1</sup>APHP, Hôpital Européen Georges Pompidou, Paris, France, <sup>2</sup>INSERM U970 Paris Cardiovascular Research Centre, Paris, Austria, <sup>3</sup>Centre de Référence des Maladies Vasculaires Rares, Paris, Austria, <sup>4</sup>INSERM U970 Paris Cardiovascular Research Centre, Paris, France.

**Introduction:** Ehlers-Danlos syndrome (EDS) is a phenotypically and molecularly heterogeneous group of inherited connective tissue disorders that share joint hypermobility, skin extensibility, abnormal scarring and tissue fragility. Vascular EDS (vEDS), the most severe EDS characterized by arterial fragility, is caused by COL3A1 mutations. Analysis of COL3A1, the gene for vEDS is performed in the Genetics Department, Georges Pompidou European Hospital, Paris. Other EDS types can be responsible for arterial fragility with minor clinical criteria, but with a better vascular prognosis and a different clinical follow-up.

**Materials and Methods:** we developed a NGS gene panel for types 1 to 7, but also rarest recessive types of EDS, corresponding to 16 genes (COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL6A1, TNXB, PLOD1, FKBP14, FBLN5, ELN, ADAMTS2, CHST14, DSE, B4GALT7, and B3GALT6). Regions of interest were targeted with a custom Haloplex enrichment kit, followed by sequencing on MiSeq. The data analysis was carried out using 3 different bioinformatics pipelines. To date 42 patients with positive Villefranche criteria were analysed.

**Results:** Causal mutations were identified in 9 patients (21%) in TNXB, COL5A1, COL1A1 and COL3A1. Numerous variants of unknown significance remain to be explored and large rearrangements to be confirmed. The identification of one patient with a COL3A1 mutation and hypermobility phenotype highlights the phenotypic overlap in EDS types.

**Conclusion:** This panel provides a powerful tool for the molecular diagnosis in EDS and will help for the characterization and best knowledge of the different EDS types, but also the genetic counseling and the management of patients.

#### P04.043

##### Chronic leg ulcer associated with premature dental loss in two brothers in Ehlers-Danlos syndrome type VIII

J. Kárteszi<sup>1</sup>, A. L. Kovács<sup>2</sup>, R. Gyulai<sup>2</sup>, A. Lőcsei-Fekete<sup>1</sup>, K. Hadzsievi<sup>1</sup>, B. Melegh<sup>1</sup>,

<sup>1</sup>Department of Medical Genetics, University of Pécs, Pécs, Hungary, <sup>2</sup>Department of Dermatology, University of Pécs, Pécs, Hungary.

**Introduction:** We report two young male patients with the clinical diagnosis of Ehlers-Danlos syndrome type VIII (MIM130080), which entity is a very rare autosomal dominant disorder characterized by periodontal disease at young age and necrobiosis lipoidica diabetorum like skin alterations. Additional features like marfanoid habitus, joint hypermobility, osteoarthritis and vascular manifestations were described in literature. The striking symptom of chronic leg ulcer was also noted in one or two cases. Neither the pathomechanism nor the underlying gene has been determined so far.

**Case Report:** We performed detailed clinical phenotype analysis of our patients, who are brothers, taking into account that less than hundred patients were published until now and plenty of questions are unanswered yet. Both of our patients were attending at dermatological examination because of chronic leg ulcer to be formed on the basis of brownish plaques over the tibia. Obvious causes in the background like diabetes, arterial and venous circulation problems were excluded. Karyotype was normal. Anamnesis unraveled almost complete loss of teeth caused by periodontitis in both of them. **Discussion:** Our poster contains beside the presentation of anamnestic data,

physical examination and basic laboratory studies the results of the histological, endocrinological, radiological and immunological work up.

**Conclusion:** We confirm that chronic leg ulcer can be an important sign leading to the diagnosis of Ehlers-Danlos syndrome type VIII as it was proposed in a previous publication by Ronceray et al. (Case Reports in Dermatological Medicine, 2013).

#### P04.044

##### Molecular analysis of 26 Ellis van Creveld syndrome (EVC) families using targeted NGS, MLPA and array CGH technologies reveals high frequency of a recurrent maternal deletion of EVC.

C. Huber<sup>1</sup>, G. Baujat<sup>1</sup>, M. Zarhrate<sup>2</sup>, C. Bole-Feysot<sup>2</sup>, C. Masson<sup>3</sup>, P. Nitschke<sup>3</sup>, S. Nusbaum<sup>4</sup>, C. Francannet<sup>5</sup>, A. Megarbane<sup>6</sup>, D. Martin-Caignard<sup>7</sup>, Y. Alembik<sup>8</sup>, M. Alex-Cordier<sup>9</sup>, K. Devriendt<sup>10</sup>, A. Dieux<sup>11</sup>, L. Faivre<sup>12</sup>, M. Gonzales<sup>13</sup>, N. Laurent<sup>12</sup>, S. Manouvrier<sup>11</sup>, M. Mathieu<sup>14</sup>, A. Paris<sup>15</sup>, F. Pellaud<sup>16</sup>, L. Spacini<sup>17</sup>, S. Saunier<sup>18</sup>, V. Malan<sup>4</sup>, V. Cormier-Daire<sup>19</sup>;

<sup>1</sup>Service de Génétique, INSERM UMR 1163, Université Paris Descartes- Sorbonne Paris Cité, Institut Imagine, Hôpital Necker Enfants Malades (AP-HP), PARIS, France

<sup>2</sup>Plateforme de Génomique, Institut IMAGINE, PARIS, France, <sup>3</sup>Plateforme de Bioinformatique, Université Paris Descartes, Paris, France, <sup>4</sup>Service de Cytogénétique, Hôpital Necker-Enfants Malades, Paris, France, <sup>5</sup>Service de Génétique Médical – CHU Estaing, Clermont – Ferrand, France, <sup>6</sup>Medical Genetics Unit, Saint Joseph University, Beirut, Lebanon, <sup>7</sup>Service de Génétique, Centre Hospitalier du Mans, Le Mans, France, <sup>8</sup>Service de Génétique Médicale, Hôpital de Hautepierre, Strasbourg, France, <sup>9</sup>Service de Génétique, Hôpital femme Mère Enfant GHE, Bron, France, <sup>10</sup>Kliniekoofd Centrum Menselijke Erfelijkheid, UZ Leuven, Campus Gasthuisberg, Leuven, Belgium, <sup>11</sup>Service de Génétique Clinique, CHRU, Hôpital Jeanne de Flandre, Lille, France, <sup>12</sup>Génétique et Anomalies du Développement, EA4271, Université de Bourgogne, Dijon, France, <sup>13</sup>Service de génétique et d'Embryologie Médicales, Hôpital Armand Trousseau, Paris, France, <sup>14</sup>Service de Génétique Clinique CHRU d'Amiens- Hôpital Nord, Amiens, France, <sup>15</sup>Service de Gynécologie Obstétrique, Maison de Santé Protestante Bordeaux-Bagatelle, Talence, France, <sup>16</sup>Pôle de Biologie, Service de Pathologie, CHU de Bordeaux, Bordeaux, France, <sup>17</sup>Unita Operativa Complessa di Ostetricia e Ginecologia, Istituti Clinici di Perfezionamento, Milano, Italy, <sup>18</sup>INSERM, UMR 1163, Laboratory of Inherited Kidney Diseases, Université Paris Descartes- Sorbonne Paris Cité, Institut Imagine, Hôpital Necker Enfants Malades (AP-HP), Paris, France, <sup>19</sup>Service de Génétique, INSERM UMR 1163, Université Paris Descartes- Sorbonne Paris Cité, Institut Imagine, Hôpital Necker Enfants Malades (AP-HP), Paris, France.

Ellis-van Creveld (EVC) syndrome is characterized by short ribs, shortened tubular bones, trident aspect of the acetabular roof, post-axial polydactyly, congenital cardiac defects, dysplastic nails and teeth and labio-gingival adhesions. EVC is caused by mutations in EVC or EVC2. More recently, mutations in DYNC2LI1 or WDR35 have been reported in a few cases.

Here, we report the molecular screening of 26 EVC family (31 cases, 17 fetuses and 14 postnatal cases) by targeted NGS (called ciliome), associated with MLPA and array CGH technology.

By Ciliome analysis, we identified variants in 20/26 families (10 nonsense, 5 splice site, 1 missense, 5 deletions) in EVC (10), EVC2 (9), EVC/EVC2 (2) and DYNC2LI1 (1). In 18 cases, the absence of any variant (6/18) and the identification of i) an heterozygous variant only (5/18), ii) an "homozygous" variant confirmed only at the heterozygous state in the father (3/18), iii) deletions identified by ciliome (4/18) prompted us to perform MLPA and CGH at EVC/EVC2 loci. We identified in 6 families the same deletion of 48Kb encompassing exon 3 to 11 of EVC always inherited from the mother. Finally, in 4/26 families only one heterozygous variant was detected and in 6/26 families, neither variant nor deletion were detected in EVC/EVC2, supporting the involvement of another disease gene. Exome sequencing is in progress. By combining ciliome analysis, MLPA and array CGH, we conclude that EVC and EVC2 account for 73 % of EVC families. Our study emphasizes the high frequency of an EVC deletion of maternal origin.

#### P04.045

##### Epidemiology approach in skeletal dysplasia in France: 8-year experience by the centres of reference for rare skeletal dysplasia

C. Messiaen<sup>1</sup>, G. Baujat<sup>1</sup>, A. Ruel<sup>1</sup>, M. Cohen Solal<sup>2</sup>, V. Forin<sup>4</sup>, C. Roux<sup>5</sup>, R. Chapurlat<sup>6</sup>, C. Michot<sup>2</sup>, R. Choquet<sup>1</sup>, V. Cormier-Daire<sup>2</sup>,

<sup>1</sup>Banque Nationale de données Maladies Rares, Hôpital Universitaire Necker-Enfants Malades, APHP, PARIS, France, <sup>2</sup>Centre de Référence Maladies Osseuses Constitutionnelles (Reference Centre for Genetic Bone Diseases), Hôpital Universitaire Necker-Enfants Malades et Institut Imagine (AP-HP), PARIS, France, <sup>3</sup>Centre de Référence Maladies Osseuses Constitutionnelles (Reference Centre for Genetic Bone Diseases), Hôpital Lariboisière (AP-HP), PARIS, France, <sup>4</sup>Centre de Référence Maladies Osseuses Constitutionnelles (Reference Centre for Genetic Bone Diseases), Hôpital Trousseau (AP-HP), PARIS, France, <sup>5</sup>Centre de Référence Maladies Osseuses Constitutionnelles (Reference Centre for Genetic Bone Diseases), Hôpital Cochin (AP-HP), PARIS, France, <sup>6</sup>Centre de Référence Dysplasies Fibreuses des Os (Reference Centre for Osteofibrous dysplasia), Hôpital Edouard Herriot (HCL), LYON, France.

**OBJECTIVES:** Skeletal dysplasias (SD) are responsible for variable types of short statures, deformations and pain, leading to severe disabilities. Since

2007, all patients seen for expertise in the french Centre of Reference (CR) for skeletal dysplasia, are systematically recorded in CEMARA, database dedicated to rare diseases.

The study objective is to characterize the epidemiological data about SD in France, to better assess care services needs.

**METHODS:** The CEMARA system records for each patient a common data set, sharing its ontology accorded to the Nosology and Classification of Constitutional Skeletal Dysplasias.

CEMARA cases were reviewed for the period 2007-2015. A « case » was defined according to the status of the diagnosis assertion: confirmed, likely, unlikely. The regional prevalences were standardized.

**RESULTS:** On the 31/12/2015, 7.874 patients with SD were identified in CEMARA. The mean of annual new cases was 887. Two third were of pediatric age. The three main diagnoses were Osteogenesis Imperfecta (1120), achondroplasia (272) and fibrous dysplasia (242). 42% of the diagnosis were confirmed. The age at onset was 14% in antenatal, 28% at birth, and 43% at a later stage. The median transport time to the CR was 33 minutes (30 km). The prevalence of patients with SD at the 31/12/2015 was 18.20 per million (IC 95% [11.59 - 27.46]) in Paris region.

**CONCLUSION:** This is the first global study about SD at an almost national level. This descriptive study allows approaching the epidemiology on specific rare diseases in France.

#### P04.046

#### Application of gene panel targeted massively parallel sequencing in genetic diagnosis of patients with Epidermolysis bullosa

*M. Hýblová<sup>1,2</sup>, J. Radvánszky<sup>3,2</sup>, P. Silló<sup>4</sup>, L. Striešková<sup>1</sup>, T. Szemes<sup>1,2</sup>,*

<sup>1</sup>*Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, <sup>2</sup>Geneton Ltd, Bratislava, Slovakia, <sup>3</sup>Institute of Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>4</sup>Faculty of Medicine, Semmelweis University, Budapest, Hungary.*

**Introduction:** Epidermolysis bullosa dystrophica is severe often life and health threatening disease affecting sublamina densa of epiderma-derma junction of the skin. This disorder is caused exclusively by mutations in COL7A1 gene resulting in recessive or dominant condition. While nonsense, frameshift and splicing mutation are characteristic for recessive condition, missense mutations particularly glycine substitution can contribute to either dominant or recessive condition. Currently, more than 300 disease causing variants in COL7A1 are known.

**Materials and methods:** Mutational analysis was carried out in 11 Hungarian patients with phenotype either autosomal recessive and dominant epidermolysis bullosa dystrofica (DEB) using targeted next generation sequencing followed by variant analysis. We analysed all exons and their flanking regions of COL7A1 gene. Genomic DNA was extracted from peripheral blood leukocytes. DNA library was prepared using transposon based protocol and analysed by massively parallel sequencing on Illumina MiSeq platform. Resulting reads were mapped to human genome hg19 and data were further evaluated using GeneTalk and HGMD database.

**Results:** MPS analysis of 552 genes in patients with assumed recessive type of EB revealed pathogenic homozygotic mutation (c.425A>G, K142R) in 2 patients, which is prevalent in central Europe. In addition, previously unknown compound heterozygote frameshift, nonsense and missense mutations were found. In dominant EB patients we identified 4 novel missense glycine substitutions and 1 glycine missense mutation previously annotated as pathogenic.

**Conclusion:** Using gene panel approach and massively parallel sequencing we were able to effectively identify novel mutations in epidermolysis bullosa patients.

#### P04.047

#### Farber disease: a growing clinical cohort indicates that acid ceramidase deficiency has a broader clinical spectrum and higher incidence than previously anticipated

*A. Solyom<sup>1</sup>, C. Simonaro<sup>2</sup>, X. He<sup>2</sup>, E. Schuchman<sup>2</sup>,*

<sup>1</sup>*Plexcera Therapeutics LLC, New York, NY, United States, <sup>2</sup>Icahn School of Medicine at Mt. Sinai, New York, NY, United States.*

Farber disease (Farber lipogranulomatosis) represents a broad clinical spectrum presenting from infancy through late childhood, with joint disease (arthritis and/or contractures), subcutaneous nodules and hoarseness associated with the pro-inflammatory and pro-apoptotic characteristics of the lipid ceramide. Farber is caused by mutations in the ASAH1 gene, which lead to acid ceramidase deficiency, accumulation of ceramide, and a distinct variety of disease phenotypes, culminating in two recognized diseases: Farber disease and Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy

(SMA-PME). The prevalence of both diseases is currently unknown and awareness of them is limited due to their rarity; both are likely underdiagnosed. We have begun collecting data including genotypes, clinical, biochemical and immunologic phenotypes, on a growing cohort of over 30 living Farber patients from around the world. Our findings so far reinforce the validity of the characteristic symptoms of Farber disease (arthritis, nodules, dysphonia). However, it also reveals that there are patients who present with only one or two of these symptoms, and that the spectrum of disease includes remarkably attenuated forms with relatively little associated disability. These insights provide an indication for screening of certain pediatric and young adult polyarticular arthritis patients for acid ceramidase deficiency, and such programs are being initiated. Acid ceramidase enzyme therapy is currently under development, and Plexcera Therapeutics is working with physicians and patients to develop a natural history study framework to better understand disease progression and factors influencing the different phenotypes, as well as what benefits can be derived from currently available anti-inflammatory treatments.

#### P04.048

#### Prevalence of fibrodysplasia ossificans progressiva (FOP) in France

*R. Choquet<sup>1</sup>, C. Messiaen<sup>1</sup>, S. Bouée<sup>2</sup>, V. Cormier-Daire<sup>1</sup>, V. Jeanbat<sup>2</sup>, K. Le Quan Sang<sup>3</sup>, A. Ruel<sup>1</sup>, C. Michot<sup>4</sup>, D. Lapius<sup>4</sup>, P. Landais<sup>5</sup>, G. Baujat<sup>6</sup>,*

<sup>1</sup>*Hôpital Universitaire Necker-Enfants Malades, Paris, France, <sup>2</sup>Cemka-Eval, Bourg la Reine, France, <sup>3</sup>Centre de Référence Maladies Osseuses Constitutionnelles, Paris, France, <sup>4</sup>LapiusData Inc, Oklahoma City, OK, United States, <sup>5</sup>Université de Montpellier, Montpellier, France.*

**Introduction:** FOP is rare, severely disabling genetic disorder characterised by heterotopic ossification of muscle and soft tissues. The objective of this study was to estimate the prevalence of FOP in France using data from two national health data collection systems: registry data from CEMARA and medical/administrative data from PMSI.

**Materials and Methods:** Records in CEMARA and PMSI (for the period 2006-2012) were reviewed for evidence of FOP. Patients who either died before or were born after 01/01/2012 (prevalence date) were excluded.

**Results:** CEMARA 85 patients with FOP were identified in CEMARA. At the prevalence date, 63 had an FOP diagnosis, 10 had experienced symptoms but had not yet received an FOP diagnosis, and the remaining 12 did not have an available diagnosis date. The prevalence was therefore 1.3 cases per million. The mean age was 29, and the age distribution was as follows: 44%<20, 48% 20-60, and 8%>60.

#### PMSI

242 patients with the ICD-10 code for FOP were identified in PMSI. The mean age was 42, and the age distribution was as follows: 18%<20, 60% 20-60, and 22%>60. The prevalence of patients with the FOP code was 3.7 cases per million; however, it is unknown whether these patients have a corresponding clinical diagnosis.

**Conclusion:** This study shows a minimum prevalence of 1.3 cases per million, higher than previous studies demonstrating 0.36/M (Spain) and 0.61/M (UK). A forthcoming study linking the two data sets will help to refine the accuracy of this estimate.

#### P04.049

#### Identification by whole exome sequencing of two novel heterozygous mutations in SH3PXD2B gene in a first case of Moroccan patient with frank-ter haar syndrome.

*A. Zrhidri<sup>1</sup>, I. C. Jaouad<sup>1,2</sup>, J. Lyahyai<sup>1</sup>, L. Raymond<sup>3</sup>, G. Egéa<sup>3</sup>, M. Taoudi<sup>3</sup>, S. El Mouatassim<sup>4</sup>, A. Sefiani<sup>1,2</sup>,*

<sup>1</sup>*Centre de Génomique Humaine, Faculté de Médecine et de Pharmacie, Mohammed V University in Rabat, Rabat, Morocco, <sup>2</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, <sup>3</sup>Département de Génétique Moléculaire, Laboratoire Biomnis, Lyon, France, <sup>4</sup>Département de Génétique Moléculaire, Laboratoire Biomnis, Rabat, Morocco.*

**Introduction:** Frank-Ter Haar syndrome (FTHS), also known as Melnick Needles like syndrome, is an autosomal-recessive disorder characterized by skeletal, cardio-vascular, and eye abnormalities, such as increased intraocular pressure, prominent eyes, and hypertelorism. The most common underlying genetic defect in Frank-Ter Haar syndrome appears to be a mutation in the SH3PXD2B gene on chromosome 5q35.1. Until now, only six mutations in SH3PXD2B gene has been identified. A genetic heterogeneity of FTHS was suggested in previous studies.

**Material and Methods:** FTHS was suspected clinically in a girl of 2 years old, born from non-consanguineous Moroccan healthy parents. The patient had been referred to a medical genetics outpatient clinic for dysmorphic facial features. Whole exome sequencing was performed in the patient and their parents, in addition to Sanger sequencing that was carried out to confirm

the results obtained by whole exome sequencing.

Results: We report the first description of a Moroccan FTHS patient with two novel compound mutations c.806G>A; p.Trp269Ter (maternal allele) and c.892delC; p.Asp299ThrfsTer44 (paternal allele) in the SH3PXD2B gene. Sanger sequencing confirmed this mutation in the affected girl and demonstrated that their parents carry this mutation in heterozygous state. Conclusion: Our results confirm the clinical diagnosis of FTHS in this reported family and contribute to expand the mutational spectrum of this rare disease. Our study shows also, that exome sequencing is a powerful and a cost-effective tool for the diagnosis of a supposed genetically heterogeneous disorder such FTHS.

#### P04.050

##### A PheWAS approach in studying genital prolapse

M. B. Khadzhieva<sup>1,2</sup>, D. S. Kolobkov<sup>1</sup>, S. V. Kamoeva<sup>3</sup>, L. E. Salnikova<sup>1,2</sup>;

<sup>1</sup>N.I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russian Federation, <sup>2</sup>Federal Research Center of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev, Moscow, Russian Federation, <sup>3</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Genital prolapse (GP) is the dropping of the pelvic organs caused by weakness or damage to the normal support of the pelvic floor. We used the PheWAS (Phenome-wide association studies) Catalog for the search of SNPs associated with GP (P-value<0.05). 159 SNPs were found for the GP PheWAS group which consisted of 718 women. These SNPs were assigned to 175 genes with GSA-SNP web server. The enrichment analysis of the PheWAS Catalog gene set associated with GP was performed with KOBAS2.0 resource in comparison with the NHGRI Catalog gene-phenotype associations. We set the minimum number of genes per category to five, and Benjamini-Hochberg FDR corrected P-value<0.10. GP associations corresponded to those found for bone mineral density (BMD) (spine). The most pronounced effect in the series of six genes (*WLS*, *SP7*, *MEPE-HSP90AB3P*, *C6orf10*, *CCDC170* and *SPTBN1*) related to BMD was observed for the *SP7* gene. The *SP7* rs10876432-A allele has been associated with BMD decrease in the NHGRI GWAS Catalog and with genital prolapse ( $P=0.006243$ , OR=1.195) in the PheWAS Catalog. *SP7* controls mesenchymal-stem cell differentiation; it is a bone specific transcription factor required for osteoblast differentiation and bone formation. Relationship between lower BMD and POP is well-known, with a purported pathophysiological mechanism based on the global collagen dysfunction for both disorders. Since supporting and binding tissues of all sorts are differentiated from common precursors, the role of the genes related to skeletal progenitor differentiation in POP development is plausible.

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#### P04.051

##### Characterization of germline genotypes in MC1R, ATM and MITF genes in Spanish Giant Congenital Melanocytic nevi patients

J. A. Puig-Butillé<sup>1,2</sup>, G. Tell<sup>3,2</sup>, V. Martins da Silva<sup>3</sup>, K. Jagirdar<sup>4</sup>, M. Potrony<sup>3</sup>, D. L. Duffy<sup>5</sup>, H. P. Soyer<sup>6,4</sup>, S. Puig<sup>3,2</sup>, R. Sturm<sup>4</sup>, A. Vicente<sup>7</sup>, M. A. Gonzalez-Ensenyat<sup>7</sup>, J. Malvehy<sup>3,2</sup>;

<sup>1</sup>Biochemistry and Molecular Genetics Department, Melanoma Unit, Hospital Clínic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>2</sup>CIBERER, Instituto de Salud Carlos III (ISCIII), Barcelona, Spain, <sup>3</sup>Dermatology Department, Melanoma Unit, Hospital Clínic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>4</sup>Dermatology Research Centre, School of Medicine, The University of Queensland, Translational Research Institute, Brisbane, Australia, <sup>5</sup>QIMR-Berghofer Medical Research Institute, Brisbane, Australia, <sup>6</sup>Department of Dermatology, Princess Alexandra Hospital, Brisbane, Australia, <sup>7</sup>Clinic of Dermatology, Hospital Universitari Sant Joan de Déu, Barcelona, Spain.

Introduction: Giant congenital melanocytic nevi (GCMN) are rare melanocytic benign tumors associated with neurocutaneous melanosis and risk of melanoma and other malignancies. The genetic cause is likely to be the acquisition of postzygotic alterations. There are evidences of predisposition for GCMN development. We hypothesized that germline variants in melanoma susceptibility genes (MC1R, ATM and MITF) have a role on this predisposition.

Materials and Methods: Analysis of MC1R gene was performed in 21 patients by Sanger sequencing. Evaluation of ATM and p.E318-MITF variant was performed in 76.2% (16/21) of them by CoreExome genotype platform (Illumina). ATM variants were evaluated by ExAC Browser European frequency (EB-E freq) and functional effect (Polyphen and Shift information).

Results:

MC1R variants were detected in 57.1% GCMN patients. Statistically significant differences between patients and control population (N=296) in terms of number of alleles or type (Red hair vs non-Red hair alleles) were

not detected. However, we observed a higher prevalence of patients harboring >1 MC1R variant (19%) compare to control population (9.8%). Moreover, 18.7% (3/16) GCMN patients carry rare ATM variants: p.L1420F (EB-E freq= 0.019; benign/tolerated), p.V410A (EB-E freq= 0.003; benign/delete-rious) and p.S333F (EB-E freq= 0.001; possibly damaging/deleterious). In contrast, p.E318K-MITF variant was not detected in the set of patients.

Conclusions: The study suggests that GCMN patients have a higher incidence of MC1R variants and rare ATM variants. Study of a large number of patients is necessary to confirm such findings.

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#### P04.052

##### Cellular and molecular defects in Hermansky-Pudlak syndrome with a novel mutation in HPS5

J. Stephen<sup>1</sup>, Y. Tadafumi<sup>1</sup>, N. Tolman<sup>2</sup>, K. J. O'Brien<sup>1</sup>, B. P. Brooks<sup>3</sup>, L. Huryn<sup>3</sup>, D. Adams<sup>2,4</sup>, D. Chen<sup>5</sup>, W. A. Gahl<sup>1,2,4</sup>, M. V. Malicdan<sup>2,4</sup>, B. R. Gochuico<sup>1</sup>;

<sup>1</sup>Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States, <sup>2</sup>NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, National Institutes of Health, Bethesda, MD, United States, <sup>3</sup>Ophthalmic Genetics & Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, United States, <sup>4</sup>Office of the Clinical Director, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States, <sup>5</sup>Division of Hematopathology, Mayo Clinic, Rochester, MN, United States.

Hermansky Pudlak Syndrome (HPS) is a heterogeneous group of genetic disorders that typically manifests with oculocutaneous albinism and bleeding diathesis. HPS is associated with defects in Biogenesis of Lysosome-related Organelle Complexes (BLOCs), a unique group of proteins that function together in the formation and/or trafficking of endo-lysosomal compartments. BLOC-2, for example, consists of three proteins HPS3, HPS5 and HPS6. Here we present a patient with defective BLOC-2 due to a novel mutation in HPS5. Under NHGRI clinical protocol 95-HG-0193, we performed extensive phenotyping in a patient with mild oculocutaneous albinism, easy bruising, absent platelet dense bodies, and no pulmonary fibrosis. Targeted panel sequencing identified a novel homozygous intronic mutation (NM\_181507.1:c.285-10A>G) in HPS5 that predicted to activate a cryptic acceptor splice site. RT-PCR analysis showed an insertion of nine nucleotides in the transcript level that predicted to lead to in-frame insertion of three amino acids (p.Ser95\_Gln96insSerCysSer) in protein level. Gene expression analysis revealed 60-70% reduction of two shorter transcripts of HPS5 and significantly reduced amounts of HPS5 protein and its interacting partners (HPS3&HPS6). Western blot and immunofluorescence microscopy showed accumulation of early endosomes (EEA1) and altered expression and distribution of late (Rab7) and recycling (Rab11) endosomes and lysosomes (CD63) in patient cells compared to control, affirming pathogenicity of the mutation. Our study broadens the genetic and phenotypic data of HPS subtype 5 and confirms the distinctive role of BLOC-2 in endosome maturation.

#### P04.053

##### Pleiotropic effects of noncoding variants near EFEMP1, WT1, ADAMTS6, and EBF2 on the risk of abdominal hernias support an etiological role for collagen and elastin homeostasis in hernia susceptibility

E. Jorgenson<sup>1</sup>, N. Makki<sup>2</sup>, K. K. Thai<sup>1</sup>, D. C. Chen<sup>3</sup>, W. L. Eckalbar<sup>2</sup>, A. Avins<sup>1</sup>, N. Ahituv<sup>2</sup>;

<sup>1</sup>Kaiser Permanente Division of Research, Oakland, CA, United States, <sup>2</sup>University of California, San Francisco, San Francisco, CA, United States, <sup>3</sup>UCLA Lichtenstein Amid Hernia Clinic, Los Angeles, CA, United States.

Abdominal hernias are some of the most frequently diagnosed conditions in clinical practice, with more than twenty million hernia repair surgeries performed annually around the world. While family studies suggest that genetic factors play an important role in hernia susceptibility, studies assessing specific genetic risk factors have been limited. Because the risks associated with hernias, specifically, bowel incarceration with a substantial risk of mortality, must be balanced against the risks associated with their treatment, which include chronic pain (6%) and hernia recurrence (10%), there is a clear need for a better understanding of hernia etiology and improved treatment options. We conducted the first large-scale genetic study of hernia risk, identifying noncoding variants at four novel genetic loci underlying the risk of inguinal hernia—the most common type of hernia. We showed that four genes in these loci (EFEMP1, WT1, EBF2, and ADAMTS6) are expressed in mouse connective tissue. We investigated whether risk SNPs in these loci were associated with other abdominal hernia subtypes, specifically, umbilical, femoral, and ventral hernias. We observed significant associations between the EFEMP1 and WT1 loci and each of these subtypes, including a genome-wide significant association between EFEMP1 and ventral hernia

( $p = 7.9 \times 10^{-10}$ ). Pathway analysis suggested that WT1 and EFEMP1 might influence connective tissue maintenance/homeostasis through their action on extracellular matrix enzymes, including matrix metalloproteinases that degrade collagen and elastin fibers. This indicates that these loci affect the risk of hernias across anatomical sites and suggests their mechanism of action may underlie hernia susceptibility more generally.

#### P04.054

#### Molecular and clinical characterization of 48 patients with Hypophosphatasia

J. Tenorio<sup>1</sup>, I. Álvarez<sup>2</sup>, L. Riancho-Zarrabeitia<sup>3</sup>, G. A. Martos<sup>4</sup>, G. Mandrile<sup>5</sup>, M. de la Flor Crespo<sup>6</sup>, M. Sukchev<sup>7</sup>, M. Sherif<sup>8</sup>, I. Kramer<sup>9</sup>, M. T. Darnaude<sup>10</sup>, P. Arias<sup>11</sup>, J. Martínez-Villanueva<sup>11</sup>, R. Gómez<sup>12</sup>, J. M. Iturzaeta<sup>12</sup>, G. Otaify<sup>13</sup>, M. García-Untueta<sup>14</sup>, A. Rubinacci<sup>15</sup>, J. A. Riancho<sup>16</sup>, M. Aglan<sup>17</sup>, S. Temtamy<sup>17</sup>, M. Abdel Hamid<sup>18</sup>, J. Argente<sup>19</sup>, V. L. Ruiz-Pérez<sup>20</sup>, K. E. Heath<sup>1</sup>, P. Lapunzina<sup>1</sup>;

<sup>1</sup>Medical and Molecular Genetics Institute (INGEMM), Madrid, Spain, <sup>2</sup>Alexion Pharmaceuticals, Medical Division, Madrid, Spain, <sup>3</sup>Department of Rheumatology, Hospital Universitario Marqués Valdecilla, IDIVAL, Santander, Spain, <sup>4</sup>Department of Endocrinology, Hospital Universitario Niño Jesús, IIS La Princesa, Department of Pediatrics, Universidad Autónoma de Madrid, Madrid, Spain. Centro de Investigación Biomédica en Red (CIBERONB): Fisiopatología de la obesidad y nutrición, I, Madrid, Spain, <sup>5</sup>Medical Genetics, San Luigi University Hospital, Orbassano, Italy, University of Torino, Dept. Clinical & Biological Sciences, Torino, Italy, <sup>6</sup>Department of Pediatrics, Hospital de la Moraleja, Madrid, Spain, <sup>7</sup>Alexion Pharmaceuticals, Diagnostic specialist, Moscow, Russian Federation, <sup>8</sup>Alexion Pharma Middle East, Medical Division, Dubai Media City, United Arab Emirates, <sup>9</sup>Department of Pediatrics, Privat Hospitalet Danmark, Charlottenlund, Denmark, <sup>10</sup>Dept. of Genetics, Hospital Universitario de Móstoles, Madrid, Spain, <sup>11</sup>Department of Endocrinology, Hospital Universitario Niño Jesús, IIS La Princesa, Department of Pediatrics, Universidad Autónoma de Madrid, Madrid, Spain. Centro de Investigación Biomédica en Red (CIBERONB): Fisiopatología de la obesidad y nutrición ICSIII, Madrid, Spain, <sup>12</sup>Department of Biochemistry, Hospital Universitario La Paz. IdiPaz, Madrid, Spain, <sup>13</sup>Department of Clinical Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt, <sup>14</sup>Department of Clinical Biochemistry, Hospital Universitario Marqués Valdecilla, IDIVAL, Santander, Spain, <sup>15</sup>Bone Metabolism Unit, San Raffaele Scientific Institute, Torino, Italy, <sup>16</sup>Dept. of Internal Medicine, Hospital Universitario Marqués Valdecilla, IDIVAL, University of Cantabria, RETICEF, Santander, Spain, <sup>17</sup>Dept. of Clinical Genetics, Division of Human Genetics and Genome Research, Centre of Excellence for Human Genetics, National Research Centre, Cairo, Egypt, <sup>18</sup>Centre of Excellence for Human Genetics, National Research Centre, Department of Medical Molecular Genetics, Division of Human Genetics an

**Introduction:** Hypophosphatasia (HPP) is a rare skeletal dysplasia caused by a deficiency in the tissue non-specific alkaline phosphatase enzyme, which is encoded by ALPL. Clinical features ranges from extremely severe phenotype up to moderate adult forms. Modes of Inheritance could be either autosomal dominant or recessive.

**Material and Methods:** Patients were classified according their first clinical evidence as: i) Intra-uterine and perinatal: Perinatal HPP. ii) After birth and before 6 months of age: Infantile. iii) Between 6 months to 18 years: Childhood and iv) after 18 years old: adult. Sequencing of ALPL has been performed in 49 index patients and eleven familiars. In silico analysis for those mutations without evidence of HPP has been performed.

**Results:** 37 different mutations have been found in all the 49 patients with HPP. Additionally familial study was positive in the 11 families. Interestingly, we found in 13 patients 11 new mutations that have not been previously related to HPP yet. In silico analysis these 11 mutations have shown a clear pathogenic effect. Cosegregation analysis also supported this idea. Clinical features of these new variants included: 4-adult, 2-childhood, 4-infantile and 3-perinatal HPP.

**Conclusions:** Although ALPL has been widely studied in Hypophosphatasia, our studies highlighted the possibility to find new undescribed mutations that expands the genotype responsible for HPP and adds clinical information correlated to these new variants that is very useful for molecular diagnosis and also to follow-up patients with HPP because a new enzyme-replacement therapy for HPP has been developed.

#### P04.055

#### High diagnostic yield using next generation sequencing in patients with congenital ichthyosis

T. Skodje, J. C. Sitek, K. B. Rypdal, M. A. Kulseth, S. S. Amundsen, E. Nafstad, H. Johnsen, V. Boger, M. Kirkevold, B. Skinningsrud, Y. Sheng, Y. Strahm, L. Retterstøl; Oslo University Hospital, Oslo, Norway.

**Background:** Congenital ichthyosis encompasses a heterogenous group of skin cornification disorders. It is characterized by scaling and hyperkeratosis of the skin. Ichthyosis may be isolated or syndromic. The severity of the disease varies significantly with a clinical spectrum ranging from dry skin to life-threatening conditions. Genes involved in keratinocyte differentiation and skin barrier function are known to cause ichthyosis. A molecular dia-

gnosis establishing the genetic cause may have important clinical implications for prognosis, follow-up, and accurate genetic counseling.

Next Generation Sequencing (NGS) may be a favorable method to reach a molecular diagnosis, due to the complex features of the different ichthyoses, the lack of definite genotype-phenotype profiles, and the high number of genes involved.

**Method:** Between 2013 and 2015 26 patients clinically diagnosed with congenital ichthyosis were analyzed with NGS. All patients were investigated with a panel of 40 genes using Whole Exome Sequencing with Agilent Sure-Select capture kit on Illumina HiSeq 2500, followed by bioinformatic analysis and filtering.

**Results:** Pathogenic genetic variants providing a molecular diagnosis were identified in 17 patients (65%).

**Conclusion:** Performing NGS with a panel of 40 well-established genes seems an efficient method in clinical practice for reaching a molecular diagnosis in patients with congenital ichthyosis.

#### P04.056

#### A novel homozygous IFT122 p.I460N (c.1379T>A) mutation in Sensenbrenner syndrome: a rare disorder within two cousins.

I. Akalin<sup>1</sup>, N. Elcioglu<sup>2</sup>, Z. Gormez<sup>3</sup>, C. Candan<sup>4</sup>, S. Yilmaz<sup>1</sup>, B. Yuceturk<sup>3</sup>, H. Demirci<sup>3</sup>, P. Turhan<sup>4</sup>, B. Yuksel<sup>5</sup>, M. S. Sagiroglu<sup>6</sup>, S. Ceylaner<sup>6</sup>;

<sup>1</sup>Istanbul Medeniyet University, Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkey, <sup>2</sup>Marmara University, Faculty of Medicine, Department of Pediatric Genetics, Istanbul, Turkey, <sup>3</sup>TUBITAK, Advanced Genome & Bioinformatics Research Center (IGBAM), Kocaeli, Turkey, <sup>4</sup>Istanbul Medeniyet University, Goztepe Training and Research Hospital, Department of Pediatric Nephrology, Istanbul, Turkey, <sup>5</sup>TUBITAK, Marmara Research Center, Kocaeli, Turkey, <sup>6</sup>Intergen Genetic Diagnosis Center, Ankara, Turkey.

**Introduction:** Craniectodermal dysplasia (CED) also known as Sensenbrenner syndrome is a rare autosomal recessive heterogeneous ciliopathy primarily characterized by skeletal and ectodermal abnormalities accompanied with progressive renal failure. Genetic heterogeneity has been described with at least 4 associated genes: *IFT122* (606045), *WDR35*(613602), *IFT43*(614068) and *WDR19*(608151) on chromosomes 3q21, 2p24.1, 14q23.4 and 4p14, respectively.

**Materials and Methods:** We present two cousins with similar phenotype from both consanguineous parents. Whole exome sequencing and analysis was performed by core facility of IGBAM (TUBITAK) using Illumina HiSeq 2000/2500 and HomSI (TUBITAK, Turkey) homozygosity mapping analyses program.

**Results:** The boys (at 9 and 5 y.o. when referred) presented similar dysmorphic facial appearance (high prominent forehead, frontal bossing, telecanthus, hypertelorism, epicanthal folds, broad nasal bridge, anteverted nares, full cheeks, micrognathia and low-set prominent ears). Skeletal findings were short stature, limb shortening, short ribs, narrow chest, brachydactyly. Ectodermal findings were abnormally shaped teeth, dental fusion, hypo/microdonty, sparse hair, clinodactyly, abnormal nails and skin and joint laxity. Elder patient had progressive renal failure and transplantation is programmed. Both had normal karyotypes: 46,XY.

Using WES, we found novel homozygous missense p.I460N (c.1379T>A) mutation in *IFT122* gene that cosegregated with the disease (transcript ID: ENST00000296266 and p.I200N for transcript id: ENST00000440957). Results were Sanger confirmed.

**Conclusions:** *IFT122* is essential in cilia development and mutations were prone to Sensenbrenner Syndrome. We report a novel conserved deleterious (Polyphen2, SIFT) homozygous p.I460N (c.1379T>A) mutation unique to these consanguineous families, up to the literature.

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#### P04.057

#### Co-segregation of both dominant and recessive mode of inheritance in a novel family with IGF1R mutation

M. Willems<sup>1</sup>, F. Brioude<sup>2</sup>, C. Amouroux<sup>3</sup>, C. Jeandel<sup>3</sup>, L. Hebrard<sup>4</sup>, M. Boubal<sup>5</sup>, O. Prodhomme<sup>6</sup>, M. Al Khoury<sup>7</sup>, D. Genevieve<sup>1</sup>, I. Netchine<sup>2</sup>;

<sup>1</sup>Département de Génétique Médicale, Hôpital Arnaud de Villeneuve, Montpellier, France,

<sup>2</sup>Laboratoire de Biologie moléculaire et d'explorations fonctionnelles endocrinien, hôpital Armand Trousseau, Paris, France, <sup>3</sup>Département d'Endocrinologie Pédiatrique, Hôpital Arnaud de Villeneuve, Montpellier, France, <sup>4</sup>MPEA, Pedopsychiatrie, Hôpital Saint ELOL, Montpellier, France, <sup>5</sup>Département de Pédiatrie, Hôpital Arnaud de Villeneuve, Montpellier, France, <sup>6</sup>Département de Radiologie Pédiatrique, Hôpital Arnaud de Villeneuve, Montpellier, France, <sup>7</sup>Département de Gastro-enterologie pédiatrique Hôpital Arnaud de Villeneuve, Montpellier, France.

**Background:** Loss of function mutations in the IGF1 receptor (IGF1R) gene lead to prenatal and postnatal growth retardation associated with insulin

resistance. To date, both dominant and recessive transmissions were reported but without phenotypic expression in heterozygous carriers in families with recessive mode of inheritance.

**Methods:** We investigated a consanguineous family consisting in two parents and their four children, including the proband, referred for primordial dwarfism. She had prenatal and postnatal short stature and microcephaly below -4SD for height and OFC, moderate developmental delay, feeding difficulties, minor facial features, and normal X-rays. Two children had normal psychomotor development and height at average range. The fourth child had learning difficulties and height at -2SD. Their mother and father's heights were at -2,5 SD. Based on unusual high IGF1 levels in the proband, we performed IGF1R molecular analysis in this family.

**Results:** An homozygous missense mutation c.384T>C;p.Phe112Leu was identified in the proband. Both parents and the child having height at -2SD and learning difficulties were heterozygous, whereas both siblings having average height didn't harbour the mutation.

**Discussion:** To our knowledge, this is the first family described with phenotypic expression in both heterozygous and homozygous carriers of IGF1R mutation. It seems that each pathogenic allele confers a -2SD height and OFC drop associated with learning difficulties, leading to mild phenotype in heterozygous carriers and severe phenotype evoking primordial dwarfism in homozygote carrier. Since this diagnosis requires special care, it is necessary to identify these patients referred for primordial dwarfism by performing IGF1 dosage.

#### P04.058

#### Mosaic mutation of KITLG in a non-progressive, congenital, linear nevoid hyperpigmentation

*A. Sorlin<sup>1</sup>, A. Maruani<sup>2</sup>, Y. Duffourd<sup>1,3</sup>, P. Kuentz<sup>1,3,4</sup>, M. Chevarin<sup>1</sup>, J. St-Onge<sup>1</sup>, T. Jouan<sup>1</sup>, C. Thauvin-Robinet<sup>1,5</sup>, L. Faivre<sup>1,3,5</sup>, J. Rivière<sup>1,3,4</sup>, P. Vabres<sup>1,5,6</sup>,*

<sup>1</sup>EA4271 GAD, Université Bourgogne-Franche Comté, Dijon, France, <sup>2</sup>Service de Dermatologie, CHU de Tours, Université François Rabelais, Tours, France, <sup>3</sup>FHU TRANSLAD, CHU de Dijon et Université Bourgogne-Franche Comté, Dijon, France,

<sup>4</sup>Biologie moléculaire, Plateau Technique de Biologie, CHU Dijon, Dijon, France, <sup>5</sup>Centre de référence Anomalies du Développement et Syndromes Malformatifs, CHU Dijon, Dijon, France, <sup>6</sup>Service de Dermatologie, CHU de Dijon, Dijon, France.

Linear and whorled naevoid hypermelanosis (LWNH) consists of hyperpigmented macular swirls and streaks following Blaschko's lines. This condition has often been considered as a nonspecific manifestation of mosaicism. It has sometimes been mistaken with the pigmentary stage of incontinentia pigmenti. In a few patients, various X chromosome rearrangements have been reported, but the molecular basis of LWNH has remained unknown. We performed deep exome sequencing on skin DNA from a 6 year-old patient with congenital non-progressive linear naevoid hyperpigmentation following Blaschko's lines on his trunk and limbs, without other cutaneous or neurosensory symptoms. We identified a postzygotic heterozygous KITLG c.329A>G (p.Asp110Gly) mutation, confirmed by targeted deep sequencing in skin fibroblasts (28% of reads) and blood (18%).

KITLG (c-KIT Ligand) regulates skin pigmentation through control of melanocyte migration, proliferation and survival, and melanin synthesis. Germline KITLG mutations have been reported in patients with Familial Progressive Hyper- and Hypopigmentation (FPHH), who exhibit early-onset hyperpigmented macules, increasing in size and number until adulthood, hypopigmented macules and larger café-au-lait macules, and recently in patients with isolated hearing loss or Waardenburg syndrome type 2A (congenital hearing loss, pigmentary abnormalities of the hair, skin, and eyes). The p.Asp110Gly mutation is the first reported mosaic mutation of KITLG, in a patient with normal hearing, extending the spectrum of clinical manifestations associated with mutations in KITLG. This is also the first report of a genetic basis for LWNH, which can now - at least in this patient - be considered as a mosaic presentation of a Mendelian disorder, FPHH.

#### P04.059

#### A novel truncating LACC1 mutation in patients with rheumatoid factor-negative polyarticular juvenile idiopathic arthritis

*R. Rabionet<sup>1,2</sup>, A. Remesal<sup>3</sup>, A. Mensa-Vilaró<sup>4</sup>, S. Murias<sup>3</sup>, R. Alcobendas<sup>3</sup>, E. Gonzalez-Roca<sup>4</sup>, O. Drechsel<sup>1</sup>, E. Ruiz-Ortiz<sup>2</sup>, A. Puig<sup>1</sup>, D. Comas<sup>5</sup>, S. Ossowski<sup>1</sup>, J. Yagüe<sup>4</sup>, R. Merino<sup>3</sup>, X. Estivill<sup>1</sup>, J. I. Arostegui<sup>4</sup>,*

<sup>1</sup>Center for Genomic Regulation, Barcelona, Spain, <sup>2</sup>Dept. of Genetics, University of Barcelona, Barcelona, Spain, <sup>3</sup>Dept. of Pediatric Rheumatology, Hospital la Paz, Madrid, Spain, <sup>4</sup>Dept. of Immunology-CDB, Hospital Clinic-IDIBAPS, Barcelona, Spain, <sup>5</sup>8Departament de Ciències Experimentals i de la Salut, Institute of Evolutionary Biology. CSIC-Universitat Pompeu Fabra, Barcelona, Spain.

We observed three siblings from a Moroccan consanguineous family with an early-onset chronic and symmetric arthritis diagnosed as rheumatoid fac-

tor-negative polyarticular juvenile idiopathic arthritis (JIA). Genome-wide SNP genotyping and whole-exome sequencing (WES) were performed in affected and unaffected siblings to identify the causative gene defect.

Four homozygosity regions in chromosomes 3, 6 (n: 2) and 13 were identified as exclusively shared by affected siblings. WES identified three potential candidate variants in these regions: two amino acid substitutions, Pro86Ser in TATDN2 and Asp169Val in FARS2, and a frameshift deletion in the LACC1 gene. Genotyping of 352 healthy Moroccan individuals and bioinformatic analyses supported the frameshift c.128\_129delGT variant in the LACC1 gene, leading to a truncated protein (p.Cys43Tyrfs\*6), as the most probable causative gene defect in this family. Additional analyses of the LACC1 gene in a group of 23 Spanish sporadic patients with systemic-onset JIA revealed no additional pathogenic mutations.

Our findings show a homozygous truncating LACC1 mutation as the genetic defect underlying a severe inflammatory joint disease that segregated in a family with a recessive mode of inheritance. These evidences expand the clinical phenotype associated with LACC1 mutations to other forms of JIA than the previously described systemic-onset JIA.

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#### P04.060

#### Loeys-Dietz syndrome 2 might be caused by a new mutation in TGFBR2 gene

*A. Khmyrova, O. Agranovich, E. Mikiashvili, S. Vissarionov, V. Larionova;*  
*The Turner's Scientific and Research Orthopaedic Institute for Children, Saint-Petersburg, Russian Federation.*

The Loeys-Dietz syndrome (LDS) is autosomal dominant disease caused by mutations in TGFBR1, TGFBR2, TGFB2, TGFB3, and SMAD3 genes, characterized by systemic involvement of arterial tortuosity and aneurysms, hypertelorism, bifid uvula or/and cleft palate.

We report on 3.5-year-old male patient with clinical features of LDS. He was born with congenital clubfoot deformations and compensated hydrocephalus. He presented with craniostenosis, dolichopagiocephaly, microretrognathia high palate; hypertelorism, blue sclerae, hyperopia; patent foramen ovale of the heart; Increased elasticity of the skin, velvety texture; clinodactyly and camptodactyly of 2nd-5th fingers, flexion contracture with ulnar deviation of 3rd-5th fingers, arachnodactyly; talipes equinovarus, recurvatus knee joints; scoliosis, pectus carinatum, straightening of physiological curves of the spine, spina bifida posterior displastica S1-S2; hypermobility, dolichostenomelia, hydrocephalus. Aiming to confirm the diagnosis, we have sequenced genes known to be associated with contractual arachnodactyly, Marfan syndrome, and other congenital diseases with similar clinical features. New mutation was found in exon 6 of TGFBR2 gene (chr3: 30715721G>C) causing an amino acid substitution Arg485Pro (NM\_001024847.2). This mutation has not been described in control samples of "1000 genomes", ESP6500, and ExAC. Pathogenicity prediction algorithms defined this mutation as most likely pathogenic (SIFT: 0.000, Polyphen2\_HDIV: 1.000, Polyphen2\_HVAR: 1.000, MutationTaster: 1.000, PROVEAN: -6.820, LRT: D). Conclusions: Based on clinical features and genetic testing results we proposed that the mutation detected might be pathogenic one causing the Loeys-Dietz syndrome type 2. The proband's relatives did not demonstrate any clinical features, thus this mutation occurred de novo.

#### P04.061

#### Novel mutations in LRP6 highlight the role of WNT signaling in tooth agenesis

*C. W. Ockeloen<sup>1</sup>, K. D. Khandelwal<sup>1</sup>, K. Dreesen<sup>2</sup>, K. Ludwig<sup>3</sup>, R. Sullivan<sup>4</sup>, I. A. L. M. van Rooij<sup>1</sup>, M. Thonissen<sup>1</sup>, S. Swinnen<sup>2</sup>, M. Phan<sup>1</sup>, F. Conte<sup>1</sup>, N. Ishorst<sup>2</sup>, C. Gilissen<sup>1</sup>, L. RoaFuentes<sup>1</sup>, M. van de Vorst<sup>1</sup>, A. Henkes<sup>1</sup>, M. Steehouwer<sup>1</sup>, E. van Beusekom<sup>1</sup>, M. Bloemen<sup>1</sup>, B. Vankeirsbilck<sup>2</sup>, S. Bergé<sup>4</sup>, G. Hens<sup>2</sup>, J. Schoenaers<sup>5</sup>, V. Vander Poorten<sup>2</sup>, J. Roosenboom<sup>2</sup>, A. Verdonck<sup>2</sup>, K. Devriendt<sup>2</sup>, N. Roeveldt<sup>1</sup>, S. N. Jhangiani<sup>6</sup>, L. E. L. M. Vissers<sup>1</sup>, J. R. Lupski<sup>7</sup>, J. de Ligt<sup>2</sup>, J. W. Von den Hoff<sup>8</sup>, R. Pfundt<sup>1</sup>, H. G. Brunner<sup>1</sup>, H. Zhou<sup>1</sup>, J. Dixon<sup>4</sup>, E. Mangold<sup>3</sup>, H. van Bokhoven<sup>1</sup>, M. Dixon<sup>4</sup>, T. Kleefstra<sup>1</sup>, A. Hoischen<sup>1</sup>, C. E. L. Carels<sup>1</sup>,*

<sup>1</sup>Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>University Hospitals KU Leuven, Leuven, Belgium, <sup>3</sup>University of Bonn, Bonn, Germany, <sup>4</sup>University of Manchester, Manchester, United Kingdom, <sup>5</sup>University Hospitals KU Leuven, Leuven, Netherlands, <sup>6</sup>Baylor College of Medicine, Houston, TX, United States, <sup>7</sup>KNOW and University Medical Center Utrecht, Utrecht, Netherlands.

**Introduction:** We aimed to identify a novel genetic cause of tooth agenesis (TA) and/or orofacial clefting (OFC), both common and burdensome congenital defects, by combining whole exome sequencing (WES) and targeted re-sequencing in a large cohort of TA and OFC patients.

**Materials and methods:** WES was performed in two unrelated patients, one

with severe TA and OFC and another with severe TA only. After identifying deleterious mutations in a gene encoding the low density lipoprotein receptor-related protein 6 (*LRP6*) with WES, all its exons were re-sequenced with molecular inversion probes, in 67 patients with TA, 1,072 patients with OFC and in 706 controls.

**Results:** We identified a frameshift (c.4594delG, p.Cys1532fs) and a canonical splice site mutation (c.3398-2A>C, p.?) in *LRP6* respectively in the patient with TA and OFC, and in the patient with severe TA only. The targeted re-sequencing showed significant enrichment of unique *LRP6* variants in TA patients, but not in nonsyndromic OFC. From the 5 variants in patients with TA, 2 affect the canonical splice site and 3 were missense variants; all variants segregated with the dominant phenotype and in 1 case the missense mutation occurred *de novo*. Additional features in patients with unique *LRP6* variants were short stature (4/7), other dental anomalies (5/7) and clinodactyly (3/7).

*LRP6* acts as a co-receptor in the WNT/  $\beta$ -catenin signaling pathway. Our findings further expand the spectrum of *LRP6* phenotypes and highlight the role of WNT signaling in dental and orofacial development.

**Conclusion:** Mutations in *LRP6* cause (severe) tooth agenesis.

#### P04.062

#### Whole exome sequencing study identifies HSPG2 and MAML1 as novel candidate genes for vertebral bone marrow signal changes (Modic changes)

M. Kraatari<sup>1,2,3</sup>, S. Skarp<sup>1,2,4</sup>, J. Karppinen<sup>5</sup>, M. Männikkö<sup>1,2,4,4</sup>;

<sup>1</sup>Center for Life Course Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland, <sup>2</sup>Center for Cell – Matrix Research, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland, <sup>3</sup>Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland, <sup>4</sup>Biocenter Oulu, University of Oulu, Oulu, Finland, <sup>5</sup>Finnish Institute of Occupational Health, Health and Work Ability, and the Disability Prevention Center, Oulu, Finland.

**Introduction:** Low back pain (LBP) is a common debilitating condition. It was ranked as top cause for global years lived with disability in all developed countries. Lumbar disc degeneration (LDD) is one of the contributing factors behind low back pain (LBP). Vertebral bone marrow signal changes also known as Modic changes (MC) are a specific phenotype of LDD. MC have heritability of MC is estimated around 30% and strong association with LBP. **Materials and Methods:** Two Finnish families were studied using whole exome sequencing and traditional genotyping to identify variants co-segregating with MC. We focused on rare (MAF < 0.01) and private variants with harmful in silico predictions and variants located in regulatory regions.

**Results:** In Family I an insertion and deletion mutation in the heparan sulfate proteoglycan 2 (HSPG2) gene, resulting in a premature stop codon, cosegregated with MC. The HSPG2 encodes structural protein in mammalian cartilage and basement membranes called perlecan. Mutations in HSPG2 cause rare autosomal recessive disorders with osteochondrodysplasia. Mutations in HSPG2 have also been associated with idiopathic scoliosis. In the Family II a single nucleotide polymorphism in the MAML1 gene was identified in all affected family members. Maml1 knock out mice suffer from impaired chondrocyte maturation. Additionally MAML1 affects the activity of RUNX2 transcription factor. RUNX2 affects the osteoblast differentiation, bone formation and chondrocyte maturation and is highly expressed in the degenerated discs.

**Conclusions:** We identified two promising candidate genes for MC, HSPG2 and MAML1. Our findings are novel in lumbar spine degenerative phenotypes.

#### P04.063

#### TUFT1 - A novel susceptibility gene for metatarsophalangeal arthritis

E. Sliz<sup>1</sup>, M. Taipale<sup>1</sup>, M. Welling<sup>1</sup>, J. Ignatius<sup>2</sup>, L. Ruddock<sup>1</sup>, R. Nissi<sup>2</sup>, M. Männikkö<sup>1</sup>;

<sup>1</sup>University of Oulu, Oulu, Finland, <sup>2</sup>Oulu University Hospital, Oulu, Finland.

**Background:** Osteoarthritis (OA) is a common musculoskeletal disease characterized by degeneration of the protective cartilage layer of articulating joints. The first metatarsophalangeal (MTP I) joint is commonly affected by OA, but MTP I OA is poorly studied when compared to hip or knee OA. Nissi et al. reported a Finnish family with 13 out of 52 family members affected by early-onset MTP I OA. The aim of the present study was to identify the genetic defect(s) causing this disorder using exome sequencing.

**Methods:** Three patients and two healthy non-family members were exome sequenced. Rare variants with harmful prediction that were shared by the patients, but not found from the healthy controls, were validated by Sanger sequencing in four affected and five unaffected family members. The functional role of the identified variant was studied in vitro.

**Results:** A variant rs41310883 on TUFT1 introducing Thr175Met substi-

tution was found to co-segregate with the disease. The variant was shown to decrease TUFT1 expression at both mRNA and protein levels in HEK293 cells. Thr175Met overexpression in ATDC5 cells resulted in increased calcium content and reduced proteoglycans in ECM when compared with overexpression of wild type TUFT1 and cells with empty vector. Furthermore, ATDC5 cells overexpressing wild type and mutant TUFT1 displayed reduced expression of marker genes of chondrogenic differentiation and disrupted formation of cartilage nodules during differentiation.

**Conclusions:** We identified a novel susceptibility gene for MTP I OA. Further studies are needed to fully characterize the role of TUFT1 in the pathophysiology of OA.

#### P04.064

#### Neuromuscular disease as sole symptom in a patient with COMP-related multiple epiphyseal dysplasia: a case report

J. M. Jensen<sup>1</sup>, H. Okkels<sup>2</sup>, M. B. Petersen<sup>1</sup>, C. K. Lautrup<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark,

<sup>2</sup>Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark.

Multiple epiphyseal dysplasia (MED) is a clinically and genetically heterogeneous group of skeletal dysplasias. MED is characterized by pain particularly in hips and knee joints. Early-onset joint pain usually progress to osteoarthritic degeneration, necessitating total joint arthroplasty. A short stature, waddling gait, and enhanced fatigability are often seen. Mild childhood neuromuscular disorders have previously been reported as a presentation of MED caused by mutations in COMP.

Here, we present a striking case of a 68-year-old woman initially diagnosed with myopathy from the age of 50. Throughout life, she exhibited difficulties in more heavy physical activity due to fatigue, but otherwise she had no joint-related symptoms.

The pedigree revealed multiple members diagnosed with early onset arthritis or Legg-Calvé Perthes disease, as well as several members diagnosed with childhood or adult onset of myopathy. Within the family, numerous analyses had been conducted without final molecular diagnosis.

According to the pedigree, an autosomal dominant inheritance was strongly suspected. Molecular analysis of five MED-related genes identified a c.2152C>T mutation in the COMP gene consistent with the diagnosis of autosomal dominant MED.

To our knowledge, this is the first case of COMP-related MED with myopathy in adulthood without other MED characteristic joint symptoms. As patients with MED may be wrongly diagnosed with neuromuscular disorders, it is important to be aware of neuromuscular symptoms as a sole symptom in MED in all age groups.

#### P04.065

#### Myhre syndrome with a full spectrum of cardiovascular and gastrointestinal complications

A. M. Spinelli<sup>1</sup>, A. Morgan<sup>2</sup>, E. Rubinato<sup>1</sup>, M. C. Pellegrin<sup>3</sup>, A. Delise<sup>3</sup>, V. Guastalla<sup>3</sup>, A. Benettoni<sup>2</sup>, I. Bruno<sup>2</sup>, F. Faletta<sup>2</sup>;

<sup>1</sup>University of Trieste and University of Padova, Trieste, Italy, <sup>2</sup>Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy, <sup>3</sup>University of Trieste, Trieste, Italy.

**Introduction.** Myhre syndrome/LAPS is a rare cause of syndromic limitation of the range of joint motion, generally presenting with variable degrees of intellectual disability and short stature. Fifty-six molecularly proven cases have been reported in the medical literature to date.

**Clinical report.** The patient was born SGA at term as the only child of healthy Italian parents. Paternal and maternal ages at conception were 39 and 31 years respectively. We evaluated the girl at 14 years. Cardinal history findings were delayed motor milestones with normal speech development and a full-scale IQ of 74; mild autistic traits; stiffness gradually involving small and large joints and resulting in pronounced functional impairment of the shoulder girdle and tibiotarsal joints in her early teens; GH deficiency; thelarche variant; bilateral chronic otitis media with effusion leading to tympanosclerosis; marked retinal vessel tortuosity; astigmatism; laryngospasm during procedural sedation. Congenital heart disease (CHD) requiring surgery (tetralogy of Fallot and peripheral pulmonary stenosis) was identified at 2 weeks of age. Her long-standing hepatomegaly, already present at 6 months, and mild protein losing enteropathy might be pathogenetically related to perturbed extracellular matrix homoeostasis, as both of them were not significantly modified either by cardiac therapies or, more recently, by constrictive pericarditis. On physical examination stocky build, short palpebral fissures and skin thickening were noted. Direct sequencing of SMAD4 identified the recurrent *de novo* mutation p.Ile500Val (NM\_005359.5:c.1498A>G).

Conclusions. We report a new case of Myhre syndrome whose gastrointestinal features and unusually severe CHD broaden the clinical spectrum of the disease.

#### P04.066

#### Myhre to Marfan Syndrome: Two opposite ends of the TGF $\beta$ /BMP driven connective tissue disorder spectrum with new evidence for an abnormal fibrotic process in Myhre syndrome

*L. J. Starr<sup>1</sup>, D. Perry<sup>2</sup>, L. Y. Saka<sup>3</sup>, D. Keene<sup>3</sup>, J. W. Delaney<sup>2</sup>, J. M. Hammel<sup>2</sup>, A. Lin<sup>4</sup>, J. R. Stone<sup>4</sup>, M. E. Lindsay<sup>4</sup>, A. Haskins Olney<sup>1</sup>, J. N. Sanmann<sup>1</sup>, A. T. Yetman<sup>5</sup>*

<sup>1</sup>University of Nebraska Medical Center, Omaha, NE, United States, <sup>2</sup>Children's Hospital and Medical Center, Omaha, NE, United States, <sup>3</sup>Shriner's Hospital, Portland, OR, United States, <sup>4</sup>Mass General Hospital, Boston, MA, United States, <sup>5</sup>Children's Hospital Medical Center, Omaha, NE, United States.

Patients with either Myhre or Marfan syndrome may have devastating complications of the cardiovascular, pulmonary, and skeletal systems, but with nearly opposite characteristics. Herein we provide thorough phenotypic information on Myhre patients, including the first detailed report of advanced pathologic findings in the heart, lung, and other tissues in the less-understood Myhre syndrome. We provide evidence that an abnormal fibrotic process is occurring in these patients and provide pathologic evidence of the life-threatening abnormal wound healing that leads to restrictive disease. Marfan syndrome, caused by pathogenic mutations in *FBN1*, involves dysregulation of TGF $\beta$ /SMAD pathways and is characterized by long bone overgrowth, joint laxity, cystic lung disease and aortic dilation. Myhre syndrome, caused by mutations in one of two codons in *SMAD4*, is a condition in which patients have short long bones, compact body habitus with tightening of the joints, hearing loss and thickening of tissues. As we describe, this thickening includes cardiac and lung tissue, which can cause restrictive disease. Although undoubtedly underdiagnosed in the past, greater awareness of this condition and the availability of clinical whole exome sequencing have resulted in a rapid increase in the number of reported patients with Myhre syndrome.

We provide the first reported images and descriptions of Myhre tissue, demonstrating an abnormal healing process, which involves proliferative fibrosis and adhesions. We propose screening protocols for patients with Myhre syndrome and recommend restricting instrumentation and elective procedures for these patients to prevent significant morbidity and mortality.

#### P04.067

#### Targeted next-generation sequencing provides novel insights into the genetic basis of amelogenesis imperfecta

*M. Prasad<sup>1</sup>, H. Dolfus<sup>1,2</sup>, A. Bloch-Zupan<sup>3,4</sup>*

<sup>1</sup>Laboratoire de Génétique Médicale, INSERM U1112, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Institut de Génétique Médicale d'Alsace, Université de Strasbourg, Strasbourg, France, <sup>2</sup>Centre de Référence pour les Affections Rares en Génétique Ophtalmologique, Service de Génétique Médicale, HUS, Strasbourg, France, <sup>3</sup>Université de Strasbourg, Faculté de Chirurgie Dentaire, Hôpitaux Universitaires de Strasbourg (HUS), Pôle de Médecine et Chirurgie Bucco-dentaires, Centre de Référence des Manifestations Odontologiques des Maladies Rares, Strasbourg, France, <sup>4</sup>Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre Européen de Recherche en Biologie et en Médecine, Université de Strasbourg, CNRS UMR7104, INSERM U964, Illkirch, France.

Amelogenesis imperfecta (AI) is a clinically and genetically heterogeneous group of diseases characterised by enamel defects. To date, mutations in >20 genes have been implicated in either isolated or syndromic AI. We used a next-generation sequencing (NGS) panel targeting 585 known or candidate genes in dental disorders to screen a cohort of 61 patients with isolated and syndromic forms of AI. We were able to identify the molecular defect underlying the patients' phenotypes in 15 cases (~25%), suggesting that several additional genes mutated in AI remain to be discovered. Interestingly, mutations in *COL17A1* were the most frequent cause of isolated AI in our cohort, accounting for 8% of patients with isolated AI. Furthermore, NGS-based screening of multiple AI genes allowed us to identify a rare case of digenic inheritance in AI, with unlinked heterozygous mutations in *COL17A1* and *LAMA3* modifying the severity of the phenotype. Finally, sequencing in a seven-year-old patient presenting with isolated AI revealed the presence of a homozygous missense mutation in *CNNM4*, mutations in which result in Jalili syndrome, a diagnosis that was subsequently confirmed by ophthalmological investigation. Therefore, we demonstrate that a large proportion of the genetic heterogeneity of AI remains unsolved and that non-Mendelian inheritance patterns exist in AI. We also demonstrate that the dental clinic may be a gateway for the diagnosis and management of rare genetic disorders. This EU-funded project (ERDF) A27 „Oro-dental manifestations of rare diseases“ is supported by the RMT-TMO Offensive Sciences initiative, INTERREG IV and INTERREG V RARENET programs. [www.genosmile.eu](http://www.genosmile.eu).

#### P04.068

#### Nine novel mutations in *NF1* gene in patients with Neurofibromatosis type 1

*C. Martinez-Bouzas<sup>1,2</sup>, M. A. Lopez-Ariztegui<sup>1,2</sup>, I. Llano-Rivas<sup>1,2</sup>, M. Garcia-Barcina<sup>3</sup>, R. Saez<sup>4</sup>, H. Maortua<sup>1,2</sup>, B. Gener<sup>1,2</sup>*

<sup>1</sup>Department of Genetics, OSI Ezkerraldea-Enkarterri-Cruces, BioCruces Health Research Institute, Cruces, Vizcaya, Spain, <sup>2</sup>CCV-CIBER de Enfermedades Raras (CIBERER-ISCIII), Madrid, Spain, <sup>3</sup>Genetics Unit. OSI Bilbao-Basurto, Bilbao, Vizcaya, Spain, <sup>4</sup>Genetics Unit. OSI Donostialdea, San Sebastian, Guipuzkoa, Spain.

Neurofibromatosis type 1 (*NF1*) is a clinically heterogeneous genetic disorder characterized by café-au-lait spots, iris Lisch nodules, axillary and inguinal freckling, and multiple neurofibromas. It is caused by dominant loss of function mutations in the suppressor gene *NF1* that encodes for neurofibromin that functions as a negative regulator of RAS activity signalling.

In the present study we report clinical and molecular findings of 14 patients (6 unrelated and 7 from 3 families). We screened DNA and RNA isolated from peripheral blood leucocytes from patients that fulfil clinical criteria for *NF1*. In addition, single and multi-exon deletions/duplications screening was performed by MLPA (Kits P81, P82 and P122). The entire *NF1* gene coding region was PCR amplified from total cDNA in several overlapping fragments, and sequenced afterwards.

We identified 9 novel mutations 6 of those were *de novo*: c.1218\_1224delTCACTAT; c.1569delA; c.6752delG; c.3975-?\_8457+?del, r.1722\_1748del27 and c.7044G>A (p.Gly23478Stop). The c.6987del24 causes a frameshift mutation which co-segregates with the clinical phenotype in family one. The missense mutations c.3965A>G (p.Asp1322Gly) and c.4972A>G (p.Ile1658Val) is considered likely pathogenic *in silico* analysis and in 2 families co-segregate with the clinical phenotype in the proband and in at least one affected relative.

Besides, clinical follow-up is being performed in patient less than 4 years old with the *de novo* missense variation c.2188A>T (p.Asn730Tyr) despite it is considered likely benign *in silico* analysis who shows already five café-au-lait spots.

This study expands the database for *NF1* mutations in patients with neurofibromatosis type 1.

#### P04.069

#### The non-coding RNA, PRINS regulates IL-6 production of human keratinocytes

*J. Danis<sup>1</sup>, A. Göblös<sup>1</sup>, Z. Bata-Csörgő<sup>1,2</sup>, L. Kemény<sup>1,2</sup>, M. Széll<sup>3,2</sup>*

<sup>1</sup>University of Szeged, Dept of Dermatology and Allergology, Szeged, Hungary, <sup>2</sup>MTA-SZTE Dermatological Research Group, University of Szeged, Szeged, Hungary, <sup>3</sup>University of Szeged, Dept of Medical Genetics, Szeged, Hungary.

The previously identified non-coding RNA, PRINS, is differentially expressed in psoriatic uninvolved and healthy epidermis. Our *in vitro* experiments in keratinocytes showed that PRINS expression is altered after exposure to various stressors i. e. UVB, translation inhibition and microbial agents. A recently described potential stress signal in psoriatic involved skin may be the extracellular DNA, a potent activator of several inflammatory pathways, but its involvement in PRINS signaling was not studied before.

The aim of our study was to investigate which pathways are induced in keratinocytes by extracellular DNA, and whether PRINS is able to affects the induced inflammatory reactions, thereby its high expression in psoriatic uninvolved epidermis may contribute to disease pathogenesis.

The synthetic DNA analogue poly(dA:dT) induced PRINS expression and the production of psoriasis associated cytokines IL-6, IL-8, IL-12, IL-23 and TNF- $\alpha$ . Cytokine production was mediated through NF $\kappa$ B-, STAT3- and MAPK- pathways in keratinocytes. To study the role of PRINS in the poly(dA:dT) induced cytokine production we silenced or forced its expression by vector based methods. Robust overexpression of PRINS reduced poly(dA:dT) induced IL-6 and IL-8 production on mRNA as well as protein level, but did not affect the production of the other investigated cytokines. *In silico* analysis revealed an approximately 100 nucleotide long complementary region between PRINS and IL-6, suggesting the direct interaction between the two RNA molecules. According to our result we propose that PRINS might act as a regulator of IL-6 production through the interaction with IL-6 mRNA, leading to the degradation of it.

**P04.070****Loss-of-Function mutations in the WNT co-receptor LRP6 cause autosomal-dominant oligodontia**

**M. P. G. Massink<sup>1</sup>, M. A. Créton<sup>1</sup>, F. Spanvello<sup>1</sup>, W. M. Fennis<sup>1</sup>, M. S. Cune<sup>2</sup>, S. M. Savelberg<sup>1</sup>, I. J. Nijman<sup>1</sup>, M. M. M. Maurice<sup>1</sup>, M. van den Boogaard<sup>1</sup>, G. van Haften<sup>1</sup>,  
<sup>1</sup>University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>University Medical Center Groningen, Groningen, Netherlands.**

**Introduction:** Tooth agenesis is one of the most common developmental anomalies in man. Oligodontia, a severe form of tooth agenesis, occurs both as an isolated anomaly and as a syndromal feature.

**Materials and Methods:** We performed exome sequencing on 20 unrelated individuals with apparent non-syndromic oligodontia and failed to detect mutations in genes previously associated with oligodontia.

**Results:** In three of the probands, we detected heterozygous variants in LRP6, and sequencing of additional oligodontia-affected individuals yielded one additional mutation in LRP6. Three mutations (c.1144\_1145dupAG [p.Ala383Glyfs(\*)8], c.1779dupT [p.Glu594(\*)], and c.2224\_2225dupTT [p.Leu742Phfs(\*)7]) are predicted to truncate the protein, whereas the fourth (c.56C>T [p.Ala19Val]) is a missense variant of a conserved residue located at the cleavage site of the protein's signal peptide. All four affected individuals harboring a LRP6 mutation had a family history of tooth agenesis. LRP6 encodes a transmembrane cell-surface protein that functions as a co-receptor with members from the Frizzled protein family in the canonical Wnt/β-catenin signaling cascade. In this same pathway, WNT10A was recently identified as a major contributor in the etiology of non-syndromic oligodontia. We show that the LRP6 missense variant (c.56C>T) results in altered glycosylation and improper subcellular localization of the protein, resulting in abrogated activation of the Wnt pathway.

**Conclusions:** Our results identify LRP6 variants as contributing to the etiology of non-syndromic autosomal-dominant oligodontia and suggest that this gene is a candidate for screening in DNA diagnostics. This work was supported the Netherlands Organization for Scientific Research NWO (VICI grant to M.M.M.).

**P04.071****A novel de novo Mutation in Frizzled2 gene in a patient with an autosomal dominant Omodyplasia**

**S. Türkmen<sup>1,2</sup>, M. Spielmann<sup>2,3</sup>, N. Günes<sup>4</sup>, R. Flöttmann<sup>2</sup>, S. Mundlos<sup>2,3</sup>, B. Tüysüz<sup>4</sup>,  
<sup>1</sup>Labor Berlin Charité Vivantes, Berlin, Germany, <sup>2</sup>Charité Institute of medical Genetics, Berlin, Germany, <sup>3</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>4</sup>Department of Pediatric Genetics, Cerrahpaşa Medical School, Istanbul University, Istanbul, Turkey.**

Omodyplasia-2 (OMOD2; OMIM #164745) is a rare autosomal dominant skeletal dysplasia characterized by shortened humeri, shortened first metacarpal, and craniofacial dysmorphism including depressed nasal bridge, broad base of the nose, and long philtrum. Recently a de novo heterozygous nonsense mutation (p.Trp548\*) in the Frizzled2 (FZD2) gene was shown to cause autosomal dominant omodyplasia syndrome. Here we present the clinical and molecular genetic data of a Turkish patient with OMOD2 in whom the whole exome sequencing revealed a novel second mutation in the FZD2 gene. This is the published second mutation in the literature. This data support the findings of Haal et al and point that the heterozygous FZD2 is the cause of OMOD2.

**P04.072****Polymorphisms in the Osteoprotegerin gene are associated with bone mineral density and fracture risk in Maltese postmenopausal women**

**M. M. Formosa, A. Xuereb-Anastasi;  
 Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta.**

**Introduction:** Osteoprotegerin (OPG) is a decoy receptor for the Receptor activator of nuclear factor kappa-B ligand (RANKL), which blocks the interaction between RANKL and RANK thereby inhibiting osteoclastogenesis and bone resorption. Four polymorphisms within the OPG gene, and one polymorphism located 43kB upstream of OPG were analysed in relation to bone mineral density (BMD) and low-trauma fractures in Maltese postmenopausal women.

**Methods:** A case-control collection of 1045 women was used. Cases were women who suffered a fracture, whereas controls had no fracture history. Genotyping was performed by polymerase chain reaction followed by restriction fragment length polymorphism or real-time PCR.

**Results:** Homozygosity for the A163G minor allele G was associated with lower spine BMD (adjusted OR: 2.6 [95% confidence interval 1.0-7.0]) relative to research subjects with a normal BMD. Women carrying two copies of

the minor allele for the T950C, G1181C and rs2062377 variants had an increased fracture risk which was independent of BMD (adjusted OR: 2.7 [1.2-6.5], 2.7 [1.2-6.6], 2.4 [1.1-7.0] respectively). The same variants increased wrist and humerus fracture risk. No association with BMD or fractures was seen for the T245G variant. Haplotype-based analysis revealed that the haplotype block containing all major alleles was protective for fractures (OR: 0.7 [0.5-0.8]), whereas that containing the minor allele for the T950C and G1181C variants increased fracture risk by 3-fold, highlighting the possibility that the effect of the rs2062377 could be through linkage with these polymorphism.

**Conclusion:** OPG gene polymorphisms predispose to reduced BMD or increased fracture susceptibility in Maltese postmenopausal women.

**P04.073****Changes in telomere position effect contribute to primary osteoarthritis**

**L. Kuszel<sup>1</sup>, T. Trzeciak<sup>2</sup>, B. Begier-Krasinska<sup>3</sup>, J. Eastwood<sup>4</sup>, M. Richter<sup>2</sup>, J. Zhang<sup>5</sup>, V. Dasa<sup>6</sup>, M. Czarny-Ratajczak<sup>1,4</sup>;**

**<sup>1</sup>Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland, <sup>2</sup>Department of Orthopedics and Traumatology, Poznan University of Medical Sciences, Poznan, Poland, <sup>3</sup>Department of Hypertension, Angiology and Internal Diseases, Poznan University of Medical Sciences, Poznan, Poland, <sup>4</sup>Tulane Center for Aging, Department of Medicine, Tulane School of Medicine, New Orleans, LA, United States, <sup>5</sup>Thermo Fisher Scientific, Waltham, MA, United States, <sup>6</sup>Department of Orthopedic Surgery, Louisiana State University Health Sciences Center, New Orleans, LA, United States.**

**Introduction:** Osteoarthritis is one of the most debilitating diseases involving degeneration of the articular cartilage of joints and causing disabilities, especially in the elderly population. Genetic and epigenetic factors contribute to development of primary osteoarthritis. In this study we analyzed the consequences of telomere shortening on cartilage transcriptome in patients with osteoarthritis. The silencing effect of telomeres on genes located nearby is known as telomere position effect and the aim of our study was to determine if telomere shortening causes changes in expression of these genes. **Materials and Methods:** We analyzed patients with knee osteoarthritis and utilized unaffected and affected cartilage, collected from the same joint of each patient during total joint replacement surgery. We measured relative telomere length in chondrocytes obtained from 50 patients with qPCR. Additionally, analysis of 20 cartilage transcriptomes was completed via RNA-Seq on Ion Proton in 10 patients with osteoarthritis. RNA-Seq data were analyzed with STAR, Bowtie2, HTSeq and R.

**Results:** Analysis of relative telomere length in 50 patients with osteoarthritis revealed severe shortening of telomeres in affected cartilage compared to unaffected cartilage. Analysis of cartilage transcriptomes showed significant enrichment in differentially expressed genes from the telomere-proximal regions of all chromosomes in the cartilage with telomere shortening.

**Conclusions:** Our results show that telomere shortening affects telomere position effect and contributes to osteoarthritis pathology through changes in the cartilage transcriptome of telomere-proximal genes.

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**P04.075****What may be hidden behind a clinical diagnosis of OI**

**S. Ivanov<sup>1</sup>, R. Vazharova<sup>1,2</sup>, L. Balabanski<sup>1</sup>, S. Andonova<sup>3</sup>, M. Malinov<sup>1</sup>, A. Savov<sup>3</sup>, D. Toncheva<sup>1,4</sup>;**

**<sup>1</sup>GARH Malinov, Genomic Laboratory, Sofia, Bulgaria, <sup>2</sup>Sofia University St. Kliment Ohridski, Sofia, Bulgaria, <sup>3</sup>UHOG Maichin dom, National genetic laboratory, Medical University Sofia, Sofia, Bulgaria, <sup>4</sup>Department of Medical Genetics, Medical University Sofia, Sofia, Bulgaria.**

**Introduction:** Establishing proper diagnosis is critical for therapeutic treatment and reproductive planning of patients and families affected by rare monogenic disorders. NGS gives opportunity to shorten the way to reliable diagnosis and to enable correct genetic counseling. Here we report a rare case of male with severe X-linked hypophosphatemic rickets who was believed to be affected with Osteogenesis imperfecta (OI) for 32 years. The patient sought confirmation of the clinical diagnosis by DNA testing during the first pregnancy of his wife.

**Materials and methods:** Analysis of patient's genomic DNA was performed using TruSight One gene panel on the Illumina MiSeq system and confirmed by Sanger sequencing.

**Results:** Patient's diagnosis was believed to be Osteogenesis imperfecta type 1 based mainly on observation of 11 long bones fractures during childhood, asymmetric legs shortening and short adult height. No fractures have been observed after puberty. During one hospitalization for surgical treatment increased level of serum parathyroid hormone was registered but no further

tests have been performed. NGS testing revealed no mutations in all known genes implicated in OI, but a rare probably pathogenic missense variant in PHEX gene (p.Pro401Leu). Further laboratory tests of blood and urine confirmed diagnosis of X-linked hypophosphatemic rickets.

Conclusion: Phenotypic variability is well-known feature of many genetic disorders, may cause misdiagnosis and even mistreatment of patients. NGS technology gives opportunity to resolve at early stage such complex cases and correct patient's medical management.

#### P04.076

##### Absence of the ER cation channel *TMEM38B*/TRIC-B disrupts intracellular calcium homeostasis and dysregulates collagen synthesis in recessive osteogenesis imperfecta

*J. C. Marini<sup>1</sup>, W. A. Cabral<sup>1</sup>, M. Ishikawa<sup>2,3</sup>, E. N. Makareeva<sup>4</sup>, B. M. Sargent<sup>1</sup>, M. Weis<sup>5</sup>, A. M. Barnes<sup>1</sup>, E. A. Webb<sup>6,7</sup>, N. J. Shaw<sup>7</sup>, L. Ala-Kokko<sup>8</sup>, F. L. Lachbawian<sup>9,10</sup>, W. Höglér<sup>5,7</sup>, S. Leikin<sup>4</sup>, D. R. Eyre<sup>2</sup>, Y. Yamada<sup>2</sup>,*

<sup>1</sup>Section on Heritable Disorders of Bone and Extracellular Matrix, NICHD, NIH, Bethesda, MD, United States, <sup>2</sup>Molecular Biology Section, NIDCR, NIH, Bethesda, MD, United States,

<sup>3</sup>Present Address: Department of Restorative Dentistry, Division of Operative Dentistry, Tohoku University, Graduate School of Dentistry, Sendai, Japan, <sup>4</sup>Section on Physical Biochemistry, NICHD, NIH, Bethesda, MD, United States, <sup>5</sup>Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA, United States, <sup>6</sup>School of Clinical and Experimental Medicine, Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom, <sup>7</sup>Department of Endocrinology and Diabetes, Birmingham Children's Hospital, Birmingham, United Kingdom, <sup>8</sup>Connective Tissue Gene Tests, Allentown, PA, United States, <sup>9</sup>Department of Medical Genetics, Children's National Medical Center, Washington, DC, United States, <sup>10</sup>Present Address: Molecular Genetics Pathology Section, Department of Molecular Pathology, Robert Tomsich Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH, United States.

Introduction: Type XIV osteogenesis imperfecta (OI) is a novel form of moderately severe OI caused by null mutations in *TMEM38B*. *TMEM38B* encodes the ER membrane monovalent cation channel, TRIC-B, proposed to counterbalance IP<sub>3</sub>R-mediated Ca<sup>2+</sup> release from intracellular stores. However, the molecular mechanisms by which *TMEM38B* mutations cause OI are unknown.

Methods: We identified 3 probands with recessive mutations in *TMEM38B* and investigated calcium signaling and collagen synthesis in proband fibroblasts and osteoblasts.

Results: TRIC-B protein is undetectable in proband fibroblasts and osteoblasts, although *TMEM38B* transcripts are present at 19-86% of control levels. TRIC-B deficiency causes impaired release of ER luminal Ca<sup>2+</sup>, associated with deficient store-operated calcium entry, despite normal stability of SERCA and IP<sub>3</sub>R, the channels for ER Ca<sup>2+</sup> uptake and release, respectively. Disturbed Ca<sup>2+</sup> flux is consistent with ER stress and increased BiP. In the absence of TRIC-B, synthesis of type I collagen is dysregulated at multiple steps. Collagen helical lysine hydroxylation is reduced despite increased LH1, and telopeptide hydroxylation is increased despite decreased Ca<sup>2+</sup>-dependent FKBP65. Although PDI levels are maintained, procollagen chain assembly is delayed in proband cells. The resulting misfolded collagen is substantially retained in TRIC-B null cells, consistent with a 50-70% reduction in secreted collagen. Lower-stability forms of collagen that elude proteosomal degradation are not incorporated into extracellular matrix, resulting in matrix insufficiency.

Conclusions: These data support a role for TRIC-B in intracellular Ca<sup>2+</sup> homeostasis, and demonstrate that absence of *TMEM38B* causes OI by dysregulation of multiple Ca<sup>2+</sup>-regulated collagen-specific chaperones and modifying enzymes in the ER.

#### P04.077

##### Familial case of osteogenesis imperfecta with three variants of clinical interest.

*J. López<sup>1</sup>, S. Franco<sup>2</sup>, S. Carmona<sup>1</sup>, C. Torres<sup>1</sup>, J. Lezana<sup>1</sup>, I. Diez<sup>1</sup>, M. Ruiz<sup>1</sup>, C. Benito<sup>2</sup>, C. Sánchez<sup>1</sup>, J. López-Siles<sup>1</sup>,*

<sup>1</sup>M.G.C. Genetaq, Málaga, Spain, <sup>2</sup>Fetal-Maternal Hospital, Málaga, Spain.

Introduction. We show a consanguineous family case with diagnosis of osteogenesis imperfecta (OI). The two siblings exhibited multiple bone fractures and *valgus* foot, compatible with OI type I. No relatives had clinical features of the disease.

Materials and methods. The sibling with the most severe clinical findings was selected for the sequencing of all genes (14) associated with OI so far. Targeted genes were captured and amplified using TruSight One library and sequenced by NextSeq (Illumina).

Results. Three variants of clinical interest were detected in heterozygous state in the proband:

Gene	Variant	Accession number (HGMD / NCBI)	Classification
<i>COL1A1</i>	c.1249C>T (p.Pro417Ser) <sup>(*)</sup>	-	Variant Of Unknown clinical Significance (VOUS)
	c.3564_3572dupCCCTGGTC	CI013220	Pathogenic
<i>COL1A2</i>	c.3313G>A (p.Gly1105Ser)	CM123314/rs139851311	Likely pathogenic

<sup>(\*)</sup>Without literature information.

The familial analysis of these variants was:

- Proband's brother: the same genotype as the proband.
- Proband's father: the same genotype as the proband.
- Proband's mother: c.1249C>T and c.3313G>A. Heterozygous state.
- Paternal aunt 1: c.3313G>A. Heterozygous state.
- Paternal aunt 2: no mutations.

Conclusions. According to the evidences for the segregation and clinical findings, the mutation c.3564\_3572dupCCCTGGTC in *COL1A1* gene could be the molecular cause of the pathology, and it could be in somatic mosaicism in the proband's father. The previously considered deleterious variant c.1249C>T, according to HGMD database, and the VOUS c.3313G>A seem not to be the molecular cause of the clinical findings in this family.

#### P04.078

##### Prenatal diagnosis of the Otopalatodigital Spectrum Disorders: a case report

*M. Mijovic<sup>1</sup>, G. Cuturilo<sup>1,2</sup>, A. Miletic<sup>1</sup>, J. Rumli<sup>1</sup>, N. Borlja<sup>1</sup>,*

<sup>1</sup>University Children's Hospital, Department of Medical Genetics, Belgrade, Serbia,

<sup>2</sup>Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

Introduction: The otopalatodigital (OPD) spectrum disorders are characterized primarily by skeletal dysplasia, inherited in an X-linked manner. In recent years, the interest in the fetal phenotypes in OPD spectrum disorders has been increased. The couple has been admitted to our genetic outpatient clinic during the second pathological pregnancy. The first pregnancy was completed by giving birth to a male newborn, who died after a few days, due to poor postnatal adaptation and respiratory complications. The newborn had skeletal dysplasia with broad thumbs and cleft soft palate. The Rubinstein-Taybi syndrome had previously been diagnosed in other institution. In the second pregnancy, with male fetus, the following anomalies have been discovered by ultrasound: low-set ears, microretrognathia, widely spaced eyes, small trunk and anomalies of the thumbs, fingers and carpal bones. Mother has telecanthus, and her healthy daughter has mild hypertelorism and mandibular hypoplasia.

Materials and Methods: Genomic DNA was extracted from fetal blood sample. We performed next generation sequencing and analysis of genes related to clinical presentation observed in patient, primarily *FLNA* gene.

Results: We identified the hemizygous variant c.620C>T (NM\_001110556.1) in *FLNA* gene, which causes a substitution of aminoacid proline with aminoacid leucine in position 207. The variant has previously been reported as patogenic in cases OPD1 (OMIM:300017.0009, ClinVar).

Conclusion: Some typical radiological and craniofacial findings in OPD spectrum disorders allow prenatal diagnosis. Molecular diagnosis is useful for refining the clinical diagnosis according to the type and location of *FLNA* mutation and for completing genetic information of the family.

#### P04.079

##### Frequent genetic disorder associated with premature loss of teeth

*I. S. M. Sayed, T. Elbadry, M. I. Mostafa, M. R. Abouzaid, N. F. Hassib, H. A. Elsadek, N. M. Ahmed, M. A. Ibrahim, M. I. Mehrez,*

*Oroental Genetics department, National Research Centre, Dokki, Egypt.*

Introduction: Premature loss of teeth is one of the features of some genetic disorder. The aim of this retrospective study is to determine the most frequent genetic disorders associated with premature loss of teeth.

Materials and Methods: The patients were selected from data record of the outpatients clinic of the Oroental Genetics department, National Research Centre, Giza, Egypt over a period of three years.

Results: The most frequent genetic disorders associated with premature loss of teeth were Papillon Lefevre syndrome and Congenital insensitivity to pain with anhidrosis. One of the characteristic features of Papillon-Lefevre Syndrome is early-onset aggressive periodontitis that affects both the primary and permanent dentitions and leads to early loss of teeth. Congenital insensitivity to pain with anhidrosis is a disorder characterized by lack of sensation to painful stimuli and self mutilating behavior which results in

autoextraction and premature loss of teeth.

Conclusions: Papillon-Lefevre Syndrome followed by Congenital insensitivity to pain with anhydrosis were the most frequent genetic disorders associated with premature loss of teeth and we report that both diseases may share similar clinical pictures and they should be included in the differential diagnosis of premature loss of teeth.

#### P04.080

##### A novel homozygous nonsense mutation in the calpastatin (CAST) gene associated with peeling skin phenotype in a Turkish child

S. G. Temel<sup>1,2</sup>, H. Sarıcaoglu<sup>3</sup>, B. Turkogenc<sup>4,5</sup>, U. Kiran<sup>3</sup>, B. Erguner<sup>6</sup>, B. Yuceturk<sup>6</sup>, M. Sagiroglu<sup>6</sup>, H. Demirci<sup>6</sup>, M. Yakicier<sup>7</sup>;

<sup>1</sup>University of Near East, Faculty of Medicine, Department of Histology & Embryology, Lefkosa, Cyprus, <sup>2</sup>University of Uludag, Faculty of Medicine, Department of Histology & Embryology, Bursa, Turkey, <sup>3</sup>University of Uludag, Faculty of Medicine, Department of Dermatology, Bursa, Turkey, <sup>4</sup>Marmara University, Faculty of Medicine, Department of Medical Biology and Genetics, Istanbul, Turkey, <sup>5</sup>University of Acibadem, Acibadem Genetic Diagnostic Center, Istanbul, Turkey, <sup>6</sup>Advanced Genomics and Bioinformatics Research Group (IGBAM) TÜBİTAK BİLGEM UEKAE, Gebze/Kocaeli, Turkey, <sup>7</sup>University of Acibadem, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkey.

Peeling skin syndrome (PSS) is characterized by continuous shedding of the stratum corneum of the epidermis with onset from birth or infancy and lasting throughout life. Skin peeling can be accompanied by erythema, vesicular lesions, or other ectodermal features including fragile hair and nail abnormalities. PSS can be divided into acral and generalized PSS. We report a 5.5 year old boy who initially presented with skin fragility. He was the first-born of IVF twins from a consanguineous marriage at 33+5 week gestational age with hypotonia at birth. Clinical findings included fragile skin, woolly hair, sparse eyelashes and brows, palmarplantar punctate keratoderma, follicular hyperkeratosis, knuckle pads and cheilitis. Moreover mild cerebral atrophy and mild muscle involvement were observed on his MRI and NCV/EMG, respectively. His aunt also had similar clinical features but in milder form with in addition nail dystrophy. Exome sequencing revealed a homozygous c.544G>T (p.Glu182\*) nonsense mutation in the CAST gene. The segregation of this rare variant in the family was confirmed by Sanger sequencing. This novel stop-gain E182X variant produces a truncated protein lacking inhibitory domains II-IV. CAST is an endogenous specific inhibitor of calpain, a calcium-dependent cysteine protease. Recently, autosomal recessive loss of function mutations in CAST were described in PLACK syndrome characterized by generalized peeling skin, leukonychia, acral punctate keratoses, cheilitis, and knuckle pads. As far as we know our case is the fifth case of PLACK syndrome without leukonychia but with some additional previously unreported associated features including mild cerebral atrophy and muscle involvement.

#### P04.081

##### Epidermolysis bullosa simplex versus peeling skin syndrome

N. Nagy<sup>1,2,3</sup>, K. Farkas<sup>2</sup>, A. Suláki<sup>1</sup>, K. Tripolszki<sup>1</sup>, L. Kemény<sup>2,3</sup>, M. Széll<sup>1,2</sup>;

<sup>1</sup>University of Szeged, Dept of Medical Genetics, Szeged, Hungary, <sup>2</sup>MTA-SZTE Dermatological Research Group, Szeged, HUNGARY, Szeged, Hungary, <sup>3</sup>Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary.

Introduction: Localised epidermolysis bullosa simplex (EBS, OMIM 131800) is a rare monogenic skin disease featured by development of blisters on the hands and feet. Acral peeling skin syndrome (APSS, OMIM 609796) is a monogenic condition characterized by superficial painless peeling of the skin predominantly on the dorsal aspects of hands and feet. In this study, we investigated a Hungarian patient, whose clinical symptoms suggested the localised form of EBS.

Materials and methods: After informed consent was given, genetic investigations have been performed in order to identify the causative genetic abnormality responsible for the development of the skin symptoms.

Results: Mutation screening with direct sequencing of the coding regions and the flanking introns of the keratin 5 and keratin 14 genes detected only wild type sequences. Since the clinical symptoms of localised EBS and APSS may overlap with each other, mutation screening of the transglutaminase 5 (TGM5) gene has been also performed. Two missense mutations have been detected in heterozygous form: one is a novel mutation (p.Trp143Arg) and one is a recurrent mutation (p.Gly113Cys) of the TGM5 gene.

Conclusions: Taken together, we report that patient with clinically suspected EBS carried TGM5 mutations and in fact suffered from APSS. Our study give further insight into the understanding of the underlying genetic background of those patients whose have been diagnosed with localised EBS, but the disease-causing mutation could not be identified with the screening of the classical EBS genes.

#### P04.082

##### Clinical study of 459 polydactyly cases in China, 2010 to 2014

Y. Xiang, J. Bian, Z. Wang, Y. Xu, Q. Fu;

Shanghai Children's Medical Center, Shanghai, China.

Background: Polydactyly is one of the most common hereditary limb malformations, involving additional digits on the hands and/or feet, which is a very attractive model to appreciate clinical and genetic heterogeneity. A high level of heterogeneity in polydactyly has been identified in different regions. However, such data of the medical literature for Asian populations is relatively less.

Methods: This study was intended to shed light on the phenotypic manifestations of polydactyly in the recruited Chinese population and to characterize the medical literature on this condition. A total of 459 well-characterized polydactyly cases from Shanghai Children's Medical Center were recruited. Their phenotypes, inheritance patterns, and clinical heterogeneity were obtained from clinical medical records.

Results: It was found that 4.8% of cases were familial and 95.2% were sporadic. The proportions of preaxial and postaxial polydactyly types were 74.7% and 25.3%, respectively. In preaxial polydactyly, type I formed the overwhelming majority (95.9%). Among the postaxial polydactyly cases, type A was most prevalent at 69.8% and type B was witnessed in 30.2% of cases. Familial and sporadic polydactyly patients mainly had unilateral presentations. A total of 583 limbs with additional digits were recorded in the 459 subjects. Upper limb involvement was more common than lower, and right hand involvement was more common than left for preaxial polydactyly, and lower limb involvement was more common than upper in postaxial polydactyly.

Conclusions: This cohort adds useful clinical/epidemiological information to the polydactyly literature in the Chinese population and highlights its marked clinical heterogeneity.

#### P04.083

##### GAPO syndrome versus Cleidocranial dysplasia regarding management of pseudoanodontia

N. F. Hassib<sup>1</sup>, M. I. Mostafa<sup>1</sup>, M. A. AbdelRahmen<sup>1</sup>, H. A. ElSadek<sup>1</sup>, M. I. Mehrez<sup>1</sup>, M. A. AbdelKader<sup>2</sup>;

<sup>1</sup>National Research Centre, Giza, Egypt, <sup>2</sup>National Research centre, Giza, Egypt.

Introduction: Pseudoanodontia is considering one of the most challenging problems encountering the dental field. GAPO syndrome and Cleidocranial dysplasia anomaly are characterized by the presence of impacted teeth known as pseudoanodontia. The aim of the study is to present a case of GAPO syndrome and another case of Cleidocranial dysostosis and their suggestive dental managements done. Materials and Methods: The two cases were collected from the out patients clinic of the Oro-dental genetics department, National Research Centre, Giza, Egypt. Results: Despite, the pseudoanodontia are one of the main feature in both diseases, the ankylosed teeth present in the patient of GAPO syndrome required to be treated using removable appliance, while, an orthodontic traction intervention to pull impacted teeth and put them in their correct position in the dental arches was the suggested dental treatment with the patient having Cleidocranial dysplasia. Conclusions: Pseudoanodontia in both GAPO syndrome and Cleidocranial dysplasia required a separate and different dental management regarding teeth ankylosis, teeth position and occlusion evaluation.

#### P04.084

##### Rare modifier variants alter the severity of cardiovascular disease in Pseudoxanthoma Elasticum

E. Y. De Vilder<sup>1,2</sup>, L. Martin<sup>3</sup>, G. Lefthériotis<sup>4</sup>, P. Coucke<sup>1</sup>, A. De Paepe<sup>1</sup>, F. Van Nieuwerburgh<sup>5</sup>, O. M. Vanakker<sup>1</sup>;

<sup>1</sup>Center for Medical Genetics Ghent, Ghent University Hospital, Gent, Belgium,

<sup>2</sup>PhD Fellow of the Research Foundation - Flanders, Gent, Belgium, <sup>3</sup>Department of Dermatology, Angers University Hospital, Angers, France, <sup>4</sup>Department of Vascular Physiology and Sports Medicine, Angers University, Angers, France, <sup>5</sup>Department of Pharmaceutics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Gent, Belgium.

Introduction: Pseudoxanthoma elasticum (PXE), an autosomal recessive ectopic mineralization disorder caused by *ABCC6* and *ENPP1* mutations, is characterized by skin, ocular and cardiovascular symptoms. Due to striking phenotypic variability, modifier genes are intensively researched to improve counseling. We evaluated the collective influence of multiple rare variants on cardiovascular disease severity in PXE.

Material and Methods: Mixed effects of rare missense/nonsense variants were assessed by Whole Exome Sequencing in 12 PXE patients with an extreme cardiovascular phenotype (based on clinical presentation and vascular calcium scoring). Statistical analysis (SKAT-O and C-alpha testing) was



performed to identify new modifier genes significantly associated with the severe cardiovascular cohort and enrichment analysis was used to evaluate pathway and gene ontology features for these modifiers (Reactome Pathway Database).

**Results:** Respectively 16 (SKAT-O) and 57 (C-alpha) genes were significantly associated to the severe cohort. Top significant genes could be stratified in 3 groups - calcium homeostasis (*OTOP2*, *HCAR3*), association with vascular disease (*TOR2A*, *NLRP1*) and induction of apoptosis (*AHNAK2*, *BRWD1*) - while enriched pathways involved FGFR1 signaling (*FLG*) and gamma-carboxylation (*GCCX*, *VKORC1*), both associated with mineralization.

**Conclusion:** This study explored for the first time the cumulative effect of rare variants on the severity of cardiovascular disease in PXE, leading to a panel of candidate modifier genes. Hypothesis-free analysis revealed the most significant genes and enriched pathways to be already involved in vascular disease or mineralization, both hallmarks of PXE. This panel will aid in risk stratification and genetic counseling of PXE patients (FW014/ASP/084).

#### P04.085

##### Functional investigation of a genetic locus associated with psoriasis

**H. F. Ray-Jones, K. Duffus, A. McGovern, S. Eyre, R. B. Warren;**  
University of Manchester, Manchester, United Kingdom.

**Introduction:** Psoriasis is a complex, chronic autoimmune condition with a genetic component. Recent genome-wide association studies have identified several psoriasis-associated loci in intergenic regions whose function is not well understood. Here, bioinformatic and molecular biology techniques were used to identify potentially disease-causing variants and their target genes in an intergenic risk locus at 9q31.

**Materials and methods:** The 9q31 locus was characterised using several available datasets in order to (1) identify all SNPs in tight linkage disequilibrium with the lead SNP, rs10979182, and (2) search for regulatory features within the SNP set. Chromatin immunoprecipitation (ChIP) and chromosome conformation capture (3C) were used to confirm the presence of regulatory SNPs and identify gene targets, respectively, in a keratinocyte cell line (HaCaT).

**Results:** 90 SNPs were highly correlated with rs10979182 ( $r^2 > 0.8$ ) and intersected three regions clearly displaying marks of enhancers, H3K4me1 and H3K27ac, in several cell types according to ENCODE. ChIP confirmed that three SNPs in these regions also bind to these histone marks in HaCaT cells. Preliminary 3C experiments suggested the presence of long-range DNA interactions between intergenic regions and the candidate gene *KLF4*, but further tests are required to confirm if the psoriasis-associated SNPs are involved in these interactions.

**Conclusions:** This work has identified SNPs in the 9q31 locus that have a potential regulatory function in psoriasis. Further 3C analyses, alongside other functional techniques, must be conducted to identify which genes are likely to be affected by these SNPs.

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#### P04.086

##### Analyses of candidate genes and genotype-phenotype analyses in pustular psoriasis

**V. Oji<sup>1</sup>, R. Mössner<sup>2</sup>, D. Wilsmann-Theis<sup>3</sup>, P. Schulz<sup>4</sup>, A. Körber<sup>5</sup>, J. Prinz<sup>6</sup>, R. Renner<sup>7</sup>, K. Schäkel<sup>8</sup>, K. Peters<sup>9</sup>, S. Philip<sup>10</sup>, K. Reich<sup>11</sup>, L. Ebertsch<sup>12</sup>, A. B. Ekici<sup>12</sup>, S. Uebel<sup>12</sup>, C. Thiel<sup>12</sup>, M. Sticherling<sup>7</sup>, H. Traupe<sup>1</sup>, U. D. Häffnermeier<sup>12</sup>;**

<sup>1</sup>Department of Dermatology, University of Münster, Münster, Germany, <sup>2</sup>Department of Dermatology, Georg-August-University Göttingen, Göttingen, Germany, <sup>3</sup>Department of Dermatology and Allergy, University Bonn, Bonn, Germany, <sup>4</sup>Department of Dermatology, Fachklinik Bad Bentheim, Bad Bentheim, Germany, <sup>5</sup>Department of Dermatology, University of Essen, Essen, Germany, <sup>6</sup>Department of Dermatology and Allergology, Ludwig-Maximilian University Munich, Munich, Germany, <sup>7</sup>Department of Dermatology, University Hospital Erlangen, Erlangen, Germany, <sup>8</sup>Department of Dermatology, University Hospital Heidelberg, Heidelberg, Germany, <sup>9</sup>Department of Dermatology and Allergology, Hospital Bayreuth, Bayreuth, Germany, <sup>10</sup>Department of Dermatology, University of Berlin, Berlin, Germany, <sup>11</sup>Department of Dermatology, Dermatology Hamburg, Hamburg, Germany, <sup>12</sup>Human Genetics, University of Erlangen, Erlangen, Germany.

Mutations in *IL36RN*, *CARD14* and *AP1S3* have been implicated in different pustular psoriatic manifestations, especially in generalized pustular psoriasis (GPP). We recruited 51 GPP patients, 3 patients with acute generalized exanthematous pustulosis (AGEP) and 4 patients with acrodermatitis continua Hallopeau (ACH), and screened them for qualitative and quantitative changes in *IL36RN*, *CARD14* and *AP1S3* as well as for point mutations in further 4 genes coding for other members of the IL-36-pathway.

Within the GPP cohort, we identified 13 homozygous/ compound-hetero-

zygous carriers of mutations (25.5%) and 3 carriers of a single mutation (5.9%) in *IL36RN*. Carriers of two mutations had a significantly lower age of onset than non-carriers ( $p < 0.01$ ). Concomitant skin/ mucous manifestations as well as a continuous course of disease were less often observed in carriers of two *IL36RN* mutations than in non-carriers, while there was no difference in frequency of psoriatic arthritis. Two of the four ACH patients carried one *IL36RN*-mutation. We identified 4 GPP patients that carried heterozygous missense variants in *CARD14* predicted/ previously shown to lead to a change in NF- $\kappa$ B levels. Allele-frequency of two *AP1S3* mutations was similar in pustular psoriasis (2.6%) and in normal individuals (2.3%). We did not observe any evidence for quantitative changes or point mutations in genes coding for other members of the IL-36-pathway in any of the 58 patients.

Our analyses point to further genetic overlap between pustular psoriatic manifestations and indicate a specific phenotype in *IL36RN*-caused generalized pustular psoriasis.

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#### P04.087

##### The molecular basis of a cluster of Pycnodynostosis in a region of the Brazilian Northeast and the analysis of the spreading of the CTSK mutations from a highly inbreeding region

**T. F. Araujo<sup>1</sup>, E. M. Ribeiro<sup>2</sup>, A. P. Arruda<sup>3</sup>, C. A. Moreno<sup>4</sup>, D. P. Cavalcanti<sup>5</sup>;**

<sup>1</sup>State University of Campinas, Campinas, Brazil, <sup>2</sup>Hospital Infantil Albert Sabin, Fortaleza, Brazil, <sup>3</sup>Faculdade de Medicina de Juazeiro do Norte, Campinas, Brazil.

Pycnodynostosis is a rare AR disease classified among the sclerosing skeletal dysplasias. Despite its estimated low prevalence (1 per million), in the last years we found 27 affected individuals (22 families) in the Ceará State (CE) at the Brazilian Northeast, giving a local prevalence of 3 per million (population of CE: 8,904,459 inhabitants). This cluster prompted us to genotype the affected individuals, under the work hypothesis of a possible founder effect. This investigation also included 15 families from other Brazilian regions. We have studied 39 individuals (33 families), of which 18 were from Ceará. The sequencing of the CTSK by the Sanger method identified six different mutations, being five previously described, and a novel one - W29Mfs\*10. This mutation appears to be concentrated in the northwest of the Ceará. The molecular analysis associated with the investigation of the origin of the parental families allowed us to the following conclusions. 1 - The high frequency of Pycnodynostosis in the CE is a consequence of the high inbreeding, rather than a founder effect. 2 - The analysis of the origin of the families showed that the most of the found mutations in the other Brazilian regions came from Northeast by a process of intern migration of the population. 3 - The haplotype study reinforced the hypothesis of founder effect related to the novel mutation. 4 - The high frequency of homozygous mutations among the patients from CE (close to 80%) suggests that the real inbreeding is higher than the referred consanguinity (33%).

#### P04.088

##### Analysis in Chilean population of the contribution to the rheumatoid arthritis susceptibility of the marker SNPs previously described by GWAS in European and Asian populations

**P. Castro-Santos, B. Assor-Cordones, G. Essmann-Antonio, M. Yáñez-Martínez, R. Díaz-Peña;**

Universidad Autónoma de Chile, Talca, Chile.

**Introduction:** Rheumatoid arthritis (RA) is an autoimmune inflammatory rheumatic disease that affects many tissues and organs, mainly synovial joints. Like many other autoimmune diseases, RA exhibits a multifactorial etiology in which genetic and environmental factors have been established. The aim of this study was the analysis of previously described associations in GWAS developed in Caucasian and Asian populations in a Chilean population.

**Materials and Methods:** We carried out a high-density genotyping study in candidate genes, evaluating their association with RA in Chilean population accomplishing the ACR 1987 criteria (313 cases/487 controls). In total, we have genotyped 128 SNPs using the OpenArray® TaqMan platform (Applied Biosystems Inc.). Additionally, we have identified the HLA-DRB1 alleles using the commercial typing kit for Luminex HLA-DR SSO (Tepnel Lifecodes). Statistical analyses were made using the software PLINK and Haplovie. For HLA-DRB1 alleles association with RA susceptibility testing we used the statistical software IBM SPSS Statistics 21.

**Results:** We have evaluated SNPs from previous GWAS (SNPs selection from the literature), including also haplotype-tag (ht)-SNPs located inside the regions of some candidate genes (PTPN22, CTLA4, TNFAIP3, CCR6, STAT4

and PADI4). In general, significance values of the associations described in GWAS are moderate, suggesting the existence of genetic particularities in Chilean, and probably other Latin-American populations.

Conclusions: Our results support the existence of some genetics factor for RA risk shared with Caucasian and Asian population but also the presence of some differential genetic characteristics of the RA Chilean population.

## P04.089

### A 34-year-old girl with PEX7-related rhizomelic chondrodysplasia punctata

*L. Gigante<sup>1,2</sup>, E. Pisaneschi<sup>3</sup>, F. C. Sangiuliano<sup>4</sup>, A. Novelli<sup>3</sup>, T. Levade<sup>5</sup>, F. Brancati<sup>6,7</sup>;*

<sup>1</sup>*Division of Cardiology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy,*

<sup>2</sup>*Humanitas Clinical and Research Center, Milano, Italy, <sup>3</sup>Laboratory of Medical Genetics,*

*Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy, <sup>4</sup>Genetics Section, Department*

*of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy,*

<sup>5</sup>*Laboratoire de Biochimie Métabolique, Institut Fédératif de Biologie, and INSERM*

*UMR1037, CRCT, Toulouse, France, <sup>6</sup>DSMOB Department Gabriele D'Annunzio University*

*of Chieti-Pescara, Chieti, Italy, <sup>7</sup>Medical Genetics Unit Policlinico Tor Vergata University*

*Hospital of Rome, Rome, Italy.*

Rhizomelic Chondrodysplasia Punctata type 1 (RCDP1) is a peroxisome biogenesis disorder caused by mutations in PEX7 gene encoding a peroxisomal matrix proteins receptor with type 2 peroxisome targeting signal (PTS2). Punctate calcifications in epiphyseal cartilages and coronal clefts of vertebral bodies, cataract, intellectual disability and growth retardation characterized RCDP1. Most patients die before 10 years for respiratory distress or cardiac malformations. Less than 20% survive 12 years, and single patients beyond 20 years are reported.

We describe a 34 year-old girl, second child of healthy parents from a small Italian village. Pre and post-natal growth restriction, congenital bilateral cataract, seizures, absence of pituitary gland with GH deficiency and mitral insufficiency with atrial regurgitation were recorded. At clinical examination she presented with severe intellectual disability, shortening of long bones with prominent proximal involvement, spasticity, keratosis pilaris and scaling skin. RCDP1 was suspected but skeletal X-rays of childhood were not available.

Peroxisomal function evaluation revealed reduced red blood cell plasmalogen, increased plasmatic phytanic acid concentration, and normal levels of very long-chain fatty acids. PEX7 molecular testing demonstrated a homozygous c.653C>T (p.Ala218Val) mutation, a recurrent mutation previously associated to milder RCDP1.

We report clinical, biochemical and molecular features of one of the oldest ever-reported patients with RCDP1, the first with congenital pituitary hypoplasia and GH deficiency. Interestingly, mutations in PEX7 can also cause an atypical phenotype with longer survival and milder neurologic involvement, normal or near-normal growth, and absence of rhizomelia. More studies are needed to clarify the mechanisms underlying clinical variability.

## P04.090

### What is the etiology of acanthosis nigricans in SADDAN syndrome?

*I. T. Perva<sup>1</sup>, M. Puiu<sup>2</sup>, R. A. Popp<sup>3</sup>, A. Chirita-Emandi<sup>2</sup>;*

<sup>1</sup>*University of Medicine & Pharmacy Victor Babes, Timisoara, Romania, <sup>2</sup>University of*

*Medicine & Pharmacy Victor Babes, Genetics Department, Center of Genomic Medicine,*

*Timisoara, Romania, <sup>3</sup>Medical Genetics, Molecular Science Department, Iuliu Hațieganu*

*University of Medicine and Pharmacy, Cluj-Napoca, Romania.*

Background: SADDAN syndrome is a rare severe form of achondroplasia associating developmental delay and acanthosis nigricans.

Aim: To present a case report and perform a literature search to understand the aetiology of the severe AN, within the syndrome. Method: The child had history and clinical assessment, laboratory work-up (for the aetiology of AN), genetic testing and imaging to evaluate foramen magnum and cranium.

Results: A 7 year old boy, known with achondroplasia phenotype from birth, presents with specific dysmorphic features of achondroplasia, severe obesity (BMI=33.9 kg/m<sup>2</sup>, >3SD WHO reference), scoliosis in the thoracic and lumbar spine, cervical, axillary and arm folds acanthosis nigricans and linguistic delay, with difficulties adjusting to normal school curriculum. He had normal values of glucose, insulin (HOMA index 0.93), Hb1C and IGF-I. Hypothalamic-pituitary axis assessment was normal. Other lab work were unremarkable. CT of the neck and cranium revealed slightly reduced foramen magnum and mild hydrocephalus (Evans index=32). The PCR-RFLP test showed a heterozygotic G1138A mutation in FGFR3 gene. The clinical, paraclinical workup, genotype and imagistics, suggested the diagnoses of SADDAN syndrome and severe obesity. Diet and lifestyle changes were recommended. The genetic cause for this severe form of achondroplasia is not known.

Conclusions: A cause for the severe AN was not established in this case. Further assessment should investigate malignant causes for acanthosis nigricans and a specific genotype correlated with this phenotype.

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## P04.091

### WES approach for an early diagnosis of Dyggve-Melchior-Claussen syndrome in a pair of siblings with skeletal defects, severe intellectual disability and cerebral abnormalities

*M. Dentici, M. Niceta, F. Pantaleoni, M. Tartaglia, B. Dallapiccola;*

*Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy, Rome, Italy.*

Dyggve-Melchior-Claussen syndrome (DMCs) is a rare skeletal dysplasia belonging to the group of the Spondyloepimetaphyseal dysplasia (DSME), characterized by progressive short-trunk dwarfism, short barrel chest, microcephaly and mild/moderate intellectual disability (ID). Skeletal radiographic features include flattening of vertebral bodies, barrel chest and atlantoaxial instability. Mutations have been identified in the DYM gene with an autosomal recessive inheritance.

We report two male dizygotic twins first evaluated at 4 years of age with severe short stature (-4.6SD and -6.9SD, respectively), microcephaly (-5SD), severe ID. In both siblings radiologic findings enclosed generalized platyspondyly with minimal notches and anterior pointing of the vertebral bodies, rhizomelic limb shortening. Both siblings have cerebral abnormalities (hypoplastic corpus callosum, lateral ventricular dilatation and one sibling presents cerebellar vermic hypoplasia with secondary dilatation of the IV ventricle). Differential diagnosis was discussed among DSME group arguing that cerebral malformations are not typically reported in the DSME. Moreover, the typical barrel chest of DMCs was not clinically evident at our first evaluation.

We performed WES (Illumina platform HiSeq 2000), on the siblings samples using an in-house implemented pipeline. The analysis identified in both patients a homozygous known mutation in the DYM gene (c.1877delA; p.Lys626AsnfsX720), for which predictive studies suggested loss of function of the relative protein. The variant was confirmed with Sanger sequencing. This study prompted to an early diagnosis of DMCs allowing an earlier access to a long term care, crucial for the progressive orthopaedic complications that, at the time of the diagnosis, were not fully concomitant in our patients.

## P04.092

### From phenotype to genotype and back again: effective whole exome sequencing in patients with skeletal dysplasia

*P. Louro<sup>1,2</sup>, A. Beleza<sup>1</sup>, S. Lillis<sup>3</sup>, M. Jansson<sup>3</sup>, J. Ahn<sup>4</sup>, K. Ryan<sup>3</sup>, A. Calder<sup>5</sup>, C. Hall<sup>5</sup>, A.*

*Offiah<sup>6</sup>, M. Simpson<sup>7</sup>, S. Yau<sup>3</sup>, S. Mohammed<sup>8</sup>, M. Irving<sup>1,7</sup>,*

<sup>1</sup>*Department of Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>2</sup>Medical Genetics Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, <sup>3</sup>Genetics Laboratories, Viapath, London, United Kingdom, <sup>4</sup>Genetics Laboratories, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,*

<sup>5</sup>*Radiology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, <sup>6</sup>Academic Unit of Child Health, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom, <sup>7</sup>Division of Genetics and Molecular Medicine, King's College London School of Medicine, London, United Kingdom.*

Background: Skeletal dysplasia (SD) is a genetically heterogeneous group of rare diseases. The clinical diagnosis of a specific SD condition may be accomplished by a clinician with expertise with the aid of a skeletal survey. However, it is frequently challenging to make a diagnosis without accompanying molecular testing. Thus, our purpose is to highlight the clinical utility of whole exome sequencing (WES) as a diagnostic tool for SD.

Methods: Eighteen suspected SD patients were selected, including cases of osteogenesis imperfecta type 1, osteopetrosis, spondylocostal dysostosis and spondyloenchondromatosis. WES was performed using Agilent SureSelectXT Human All Exon V5 followed by sequencing on an Illumina HiSeq 2500. Raw sequence data was aligned using Novoalign, and variants called with Samtools. Qiagen Ingenuity Variant Analysis was used to aid assessment of variant pathogenicity after applying a virtual panel of 220 SD genes in combination with multidisciplinary clinical interpretation.

Results: Pathogenic variants were detected in six cases, while in four cases variants were identified that require additional work to clarify their pathogenicity. Of the remaining eight cases with no detected pathogenic variants in the selected SD genes, clinical reassessment suggests that three are unlikely to have SD.

Conclusions: WES analysis provided a definitive diagnosis in at least one third (6/18) of our selected SD patients. These results demonstrate WES

analysis using targeted gene analysis is an effective diagnostic tool for SD. However, it also emphasizes the importance of a thorough clinical evaluation and close analytical liaison with clinical scientists to provide an optimal and efficient service.

#### P04.093

#### Sweet's syndrome in a patient with compound heterozygous mutations in the Mediterranean fever gene (MEFV)

*M. Michelson Kerman, Chana Vinkler, Dorit Lev;  
Wolfson Medical Center, Holon, Israel.*

Sweet's syndrome (SS) is a rare disorder that often occurs in association with other systemic diseases.

The disorder is characterized by development of nonpruritic, painful erythematous plaques with pseudovesicles, pustules and rare bullae. SS consists of a triad of erythematous plaques infiltrated by neutrophils in association with fever and leukocytosis. The pathological features of SS involve the dermis.

The treatment of choice are systemic corticosteroids.

We present an unusual course of SS in a man who carries compound heterozygous mutations in the MEFV gene.

A 38 year old man from Sephardic Jewish ancestry, had suddenly developed fever, malaise, artralgia and painful erythematous plaques with pustules and bullae on the upper extremities. Diagnostic evaluation included leukocytosis, elevated erythrocyte sedimentation and C-reactive protein rate. The symptoms exacerbated despite treatment with systemic corticosteroids. Mutational analysis of the MEFV gene revealed compound heterozygous M694V and V726A mutations. Clinical improvement appeared after administration of colchicine.

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. The FMF gene (MEFV) encodes the protein pyrin that plays an important role in modulating the innate immune response.

MEFV mutations have been identified primarily in patients from Mediterranean populations

Sweet's syndrome has been described in a patient with classical FMF as a possible new cutaneous feature and has never been described as a presenting sign of FMF.

We suggest that SS skin lesions might be an only cutaneous presentation of FMF.

#### P04.094

#### Identification of intragenic deletions of TCF12 by whole genome sequencing

*J. A. C. Goos<sup>1</sup>, A. L. Fenwick<sup>2</sup>, S. M. A. Swagemakers<sup>1</sup>, S. R. F. Twigg<sup>2</sup>, A. J. M. Hoogeboom<sup>1</sup>, M. F. van Dooren<sup>1</sup>, F. J. Magielsen<sup>1</sup>, I. M. J. Mathijssen<sup>1</sup>, A. O. M. Wilkie<sup>2,3</sup>, P. J. van der Spek<sup>1</sup>, A. M. W. van den Ouwendal<sup>1</sup>;*

<sup>1</sup>Erasmus MC, University Medical Center, Rotterdam, Netherlands, <sup>2</sup>MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom, <sup>3</sup>Craniofacial Unit, Oxford University Hospitals NHS Trust, John Radcliffe Hospital, Oxford, United Kingdom.

**Introduction:** Coronal synostosis can be caused by heterozygous loss-of-function mutations of TCF12. Until now, however, large intragenic deletions in this gene have not been described as a cause of TCF12-related craniosynostosis. Here, we describe three large intragenic deletions in TCF12 identified by whole genome sequencing (WGS).

**Materials and methods:** Within the framework of a broader study on craniosynostosis, WGS of nineteen index-cases with coronal synostosis and their family members (forty-six samples in total) was carried out by Complete Genomics, a BGI Company (Mountain View, CA, USA). The WGS data were analyzed using an autosomal dominant disease model. Deletion-specific PCR and dideoxy-sequence analysis were performed to confirm the WGS results.

**Results:** Using WGS, three large intragenic deletions of 84.949, 8.580 and 5.363 base pairs were identified in TCF12, deleting exons 7-18, 19, and 20, respectively. The first two deletions overlap the exons in which most pathogenic point mutations have been described. All index patients had coronal suture synostosis and all deletions were inherited. However, not all parents with the deletions showed clinical signs. Similar non-penetrance has previously been described in TCF12-related craniosynostosis caused by point mutations.

**Conclusions:** Three large intragenic deletions were identified in TCF12 using WGS, indicating the importance of screening for larger rearrangements in patients suspected for TCF12-related craniosynostosis.

#### P04.095

#### TGFB1 variant and MLH1 mutation in a patient with aortic aneurysm and colon cancer

*S. Azzarello-Burri, P. Joset, A. Baumer, T. Herget, A. Rauch;  
Institute of Medical Genetics, 8952 Schlieren, Switzerland.*

Aortic aneurysms and dissections are a typical complication of connective tissue disorders such as Marfan and Loeys-Dietz syndrome, which are caused by increased TGFB signaling. Since the latter was also found in cancer, the question of increased cancer risk in patients carrying germline mutations of TGFB signaling members raised when we recently observed a patient who next to an aortic aneurysm and skeletal signs of Marfan syndrome had colon cancer at the age of 60 years. We therefore performed exome sequencing with selective analysis of known connective tissue genes and found a potentially disease causing TGFB1 variant c.553C>T/=(p.Arg185Trp/=). Mutations in the TGFB1 gene are known to cause Camurati-Engelmann disease, which is characterized by hyperostosis of the long bones and the skull, proximal muscle weakness, severe limb pain, a wide-based waddling gait, and joint contractures. Since there is one patient with Camurati-Engelmann disease and aortic dissection in his teenage years described in the literature, we assumed that the connective tissue phenotype of our patient was caused by this variant. We considered that his colon cancer might also be related to the TGFB1 variant, but for safety reason extended the exome analysis to known colon cancer genes, which indeed showed in addition a known MLH1 splice site mutation c.2103+1G>T/=. Our observation therefore indicates that despite the link between TGFB signaling and cancer development, increased cancer risk in patients with germline mutations has not been described so far and may actually be attributable to an independent second disorder.

#### P04.097

#### TRPS1 gene defects in patients with trichorhinophalangeal syndrome

*R. Solc<sup>1</sup>, K. Hirschfeldova<sup>2</sup>, M. Klugerova<sup>1</sup>, J. Vcelak<sup>3</sup>, A. Baxova<sup>2</sup>, M. Kuklik<sup>3,4</sup>, J. Vseticka<sup>5</sup>;*

<sup>1</sup>Charles University in Prague, Faculty of Science, Department of Anthropology and Human Genetics, Prague, Czech Republic, <sup>2</sup>Charles University in Prague, 1st Faculty of Medicine, Institute of Biology and Medical Genetics, Prague, Czech Republic, <sup>3</sup>Institute of Endocrinology, Department of Molecular Endocrinology, Prague, Czech Republic,

<sup>4</sup>Genetic clinic Olsanska, Prague, Czech Republic, <sup>5</sup>Genetika Ostrava s.r.o., Ostrava, Czech Republic.

**Introduction:** The aim of the study was to determine genetic defects in the group of patients with a trichorhinophalangeal syndrome (TRPS) phenotype derived from the Czech population.

**Materials and Methods:** Each of 9 probands (8 with TRPS I and 1 with TRPS II) was analyzed by use of the 228-B1 MLPA kit (MRC Holland), which covers the relevant 8q24 chromosomal region in order to detect *TRPS1* gene and *EXT1* gene rearrangements. Additionally, mutational analysis of the coding part of the *TRPS1* gene was conducted.

**Results:** In the proband with TRPS II, a large deletion was detected (about 10 Mb), which includes genes *TRPS1* and *EXT1*. In 6 of 8 probands with TRPS I we found some mutations which consider to be the causal ones. It was one large *TRPS1* intragene deletion (exons 2-5); two small structural mutations (a deletion in the exon 5, an indel mutation in the exon 4); two *nonsense* substitutions (in the exon 4 and in the exon 5); and one *missense* substitution (in the exon 6). Both the two small structural aberrations and the two *nonsense* substitutions have not been described yet. Moreover we discovered several probably polymorphic substitutions in *TRPS1* gene 3'UTR sequences. The study was supported by the Charles University in Prague, project GAUK202615.

#### P04.098

#### Sclerotic bone lesions in Tuberous Sclerosis Complex

*S. Boronat<sup>1,2</sup>, I. Barber<sup>1</sup>, E. A. Thiele<sup>2</sup>;*

<sup>1</sup>Hospital Vall d'Hebron, Barcelona, Spain, <sup>2</sup>Massachusetts General Hospital, Boston, MA, United States.

**Background:** Tuberous sclerosis complex (TSC) is due to mutations in *TSC1* or *TSC2* genes resulting in hyperactivation of the mTOR pathway. Many tissues can be affected, including brain, skin, eye, heart, bone, kidney or lung. Sclerotic bone lesions have been reported in TSC but there is scarce information about them in the literature.

**Methods:** Abdominal MR scans of 70 children with TSC who had *TSC1*/*TSC2* mutational studies were reviewed for sclerotic bone lesions, and a longitudinal study was performed in 50 patients who had two or more MR scans. Chest CT scans of 92 adult TSC patients (70 with mutational TSC studies) were reviewed for sclerotic bone lesions.

**Results:** 173 sclerotic bone lesions were detected in 51 of 70 children (73%)

and affected mainly the vertebrae pedicles. The youngest patient with these lesions was 18 months old. New lesions appeared in 50% of the patients during a follow-up period of 1 to 6 years and growth of previous lesions was documented in 37% patients. Patients with sclerotic bone lesions were more frequently females (75% vs 37%) and they had more renal involvement than patients without bone lesions (84% vs 47%). Sclerotic bone lesions were found in chest CT studies of 82 adult patients (89%). Patients without bone lesions had no mutation identified in 86%.

Conclusion: Sclerotic bone lesions are very frequently found in MR and CT studies of TSC patients. They are usually present within the first years of life, and new lesions appear with age.

#### P04.099

##### A case of Weaver Syndrome caused by a novel frameshift EZH2 mutation

*H. Simsek, B. B. Geckinli, K. Delil, M. A. Soylemez, A. Turkyilmaz, E. Arslan Ates, M. Avsar, P. Ata, A. I. Guney, A. Arman;*

*Medical Genetics Department, Marmara University Medical School, Istanbul, Turkey.*

Weaver Syndrome is a rare overgrowth syndrome characterized by prenatal/postnatal overgrowth, accelerated osseous maturation, characteristic craniofacial appearance and developmental delay. Weaver syndrome is an autosomal dominant disorder caused by heterozygous mutation in the EZH2 gene which encodes histone methyltransferase.

Our patient is a 4 year-old girl who was referred to our clinic due to speech and neurodevelopmental delay. She was born at 32nd weeks of gestation by cesarian section because of mothers' preeclampsia. Her birth weight was 3000 gr (>97P), height was 52 cm (>97P), occipitofrontal circumference was 34 cm (>97P). On physical examination, she weighed 24 kg (>97P), lengthened 117 cm (>97P), and occipitofrontal circumference was 54,5 cm (>97P). Clinical examination showed macrocephaly, a broad forehead, round face, long philtrum, hypertelorism, depressed nasal bridge, large ears, large hands and feet, tibial bowing, enlarged wrists and knees, hallux valgus, umbilical hernia and pigmented nevi on trunk. Her radiographic findings were advanced carpal bone maturation of 6 years and enlarged metaphysis of long bones. DNA sequence analysis of EZH2 gene showed a de novo novel heterozygous p.D730\* (c.2187\_2188insT) frameshift mutation in exon 19. This truncating mutation is identified at the SET domain. Identification of EZH2 gene mutations is important to clarify genotype-phenotype correlations in Weaver syndrome.

#### P04.100

##### Whole Exome Sequencing reveals a mutation in an osteogenesis imperfecta patient

*M. A. Ergun<sup>1</sup>, G. Bilgili<sup>2</sup>, U. Hamurcu<sup>3</sup>, A. Ertan<sup>4</sup>;*

<sup>1</sup>Gazi University Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Gazi University Faculty of Medicine, Department of Physiology, Ankara, Turkey, <sup>3</sup>Hamurcu Clinic, Ankara, Turkey, <sup>4</sup>Hacettepe University, Faculty of Dentistry Department of Prosthetic Dentistry, Ankara, Turkey.

Introduction: Osteogenesis imperfecta (OI) is an autosomal dominant disorder characterized mainly by bone fragility and blue sclerae. OI is caused by mutations in type I collagen genes; COL1A1 and COL1A2. Dentinogenesis imperfecta is a common disorder for osteogenesis imperfecta patients. More than half of the OI patients have also dentinogenesis imperfecta. Whole Exome sequencing (WES), involves exome capture, which limits sequencing to the protein coding regions of the genome, composed of about 20,000 genes, 180,000 exons, and constituting approximately 1% of the whole genome. A major indication for use is molecular diagnosis of patients with suspected genetic disorders or of patients with known genetic disorders with substantial genetic heterogeneity involving substantial gene complexity.

Materials and Methods: In this study, we performed WES for a patient diagnosed as Osteogenesis Imperfecta. He had also dentinogenesis imperfecta.

Results: The WES results confirmed with Sanger sequencing revealed as a missense mutation at codon 560 of COL1A1 gene: c.1678 G>A (G560S). The mutation was on exon 25 and according to the dbSNP database this mutation corresponded to rs67507747.

Conclusions: It is very important to perform WES after an algorithm. This algorithm has to include, a suspect of a mendelian disorder, multiple genetic conditions in the differential diagnosis, and even if it is available the conventional diagnosis is prohibitively expensive. Finally, Sanger sequencing in order to confirm the results is also advised.

#### P04.101

##### Role of missense variants in Wnt pathway genes in BMD determination

*N. Martinez-Gil<sup>1</sup>, N. Roca-Ayats<sup>1</sup>, R. Urreizti<sup>1</sup>, H. Franco-Valls<sup>1</sup>, N. Garcia-Giralt<sup>2</sup>, L. Mellibovsky<sup>2</sup>, X. Nogués<sup>2</sup>, A. Díez-Pérez<sup>2</sup>, D. Grinberg<sup>1</sup>, S. Balcells<sup>1</sup>;*

<sup>1</sup>Universitat de Barcelona, IBUB, CIBERER, Barcelona, Spain, <sup>2</sup>URFOA, RETICEF, IMIM, Parc de Salut Mar, Barcelona, Spain.

Osteoporosis is a common disease characterized by decreased bone mineral density (BMD) and an increased fracture risk. It is determined by environmental and additive genetic susceptibility factors. In a meta-analysis by Estrada *et al.* (2012), 56 loci were found associated with BMD, 14 of which were also associated with osteoporotic fracture. Notably, the list was enriched for genes of the Wnt pathway.

To better understand the role of Wnt pathway genes in determining osteoporosis, we aimed at exploring the allelic architecture of *SOST*, *WNT16* and *DKK1* with an emphasis in their coding regions. We resequenced all the exons and intronic flanking regions in two extreme BMD groups from the BARCOS cohort of Spanish postmenopausal women. Selected variants were then genotyped in the full cohort and tested for association to BMD.

Initially, 17, 3 and 13 single nucleotide variants were identified in *WNT16*, *SOST* and *DKK1*, respectively. Seven SNPs showing biased frequencies in the two extreme groups and 6 rare variants located in putative regulatory elements were genotyped. Significant results were obtained for an intronic SNP (rs142005327) in *WNT16* and for all the missense variants in *WNT16* (rs2908004, p.G72R/p.G82R; rs2707466, p.T253I/p.T263I) and *SOST* (rs17882143, p.V10I). The associated SNPs are in linkage disequilibrium with the respective GWAs hits. A rare variant (rs570754792, *SOST*) was found in 3 women whose BMD was below the mean of the BARCOS cohort. Our results, suggest that missense variants in *SOST* and *WNT16* are most likely to influence BMD.

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#### P04.102

##### Novel mutations in the keratin-74 (KRT74) gene underlie autosomal dominant woolly hair/hypotrichosis in Pakistani families

*N. W. Naveed<sup>1</sup>, W. Ahmad<sup>2</sup>;*

<sup>1</sup>Institut für Humangenetik, Uniklinik, Ulm, Germany, <sup>2</sup>Department of Biochemistry, QAU, Islamabad, Pakistan.

Autosomal dominant woolly hair (ADWH) is an inherited condition of tightly curled and twisted scalp hair. Recently, a mutation in human keratin-74 (KRT74) gene has been shown to cause this form of hereditary hair disorder. In the present study, we have described two families (A and B) having multiple individuals affected with autosomal dominant form of hair loss disorders. In family A, 10 individuals showed ADWH phenotype while in the family B, 14 individuals showed hypotrichosis of the scalp. Genotyping using polymorphic microsatellite markers showed linkage of both the families to type II keratin gene cluster on the chromosome 12q12-14.1. Mutation analysis of the KRT74 gene identified two novel mutations in the affected individuals of the families. The sequence analysis revealed a splice acceptor site mutation (c.IVS8-1G>A) in family A and a missense variant (c.1444G>A, p.Asp482Asn) in family B. Mutations identified in the present study extend the body of evidence implicating the KRT74 gene in the pathogenesis of autosomal dominant hair loss disorders.

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#### P05 Cardiovascular disorders

##### P05.01

##### Genetic variants in familial abdominal aortic aneurysms identified by whole genome and exome sequencing

*A. IJpma, D. Heijmans, M. Oomen, H. Brüggenwirth, I. van den Boogert, D. Majoor-Krakauer;*

*Erasmus MC, Rotterdam, Netherlands.*

Introduction: Abdominal aortic aneurysm (AAA) is a frequent disorder with a prevalence of approximately 5% in the elderly population. In 20% of the patients AAA is familial. No genetic causes for AAA have been identified so far. The goal of this study is to identify genes that play a role in the formation of abdominal aneurysms. Methods: The study includes approximately 950 AAA patients. So far we sequenced the DNA of 341 patients with a family history of abdominal aortic aneurysm. Whole genome sequencing (WGS)

was performed in 3 families (15 individuals) and whole exome sequencing (WES) was performed in 50 families (107 individuals) and 219 single AAA patients with familial disease. Prioritization of resulting variants was performed according to the following gene sets: 1. Genes in diagnostics panel as applied in the Erasmus MC in thoracic or syndromic aneurysms (n=25) 2. A broad selection of genes involved in vascular function or disease (n=4209) 3. All genes in the genome Results: We present the detailed workflow of the analysis of the genomics data, including the results so far. In 105 out of 267 families a variant in one of the set 1 genes was found. Further analysis of the set 2 and 3 genes so far led to the identification of several candidate genes that show variants in more than one AAA family and that have not been linked to AAA before.

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#### P05.02

#### Associations between selenoprotein gene variants and selenoprotein levels and aortic diseases: abdominal aortic aneurysm and aortoiliac occlusive disease

*E. Strauss<sup>1,2</sup>, J. Tomczak<sup>1</sup>, L. Wawrzynska<sup>1</sup>, R. Staniszewski<sup>1</sup>, G. Oszkinis<sup>1</sup>;*

<sup>1</sup>Department of General and Vascular Surgery, Poznan University of Medical Sciences, Poznan, Poland, <sup>2</sup>Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland.

Introduction. In this study the role of common genetic variants in selenoprotein genes (SEPP1 rs3877899, rs7579, SELS rs34713741 TXNRD1 rs35009941, TXNRD2 rs9605031, GPX4 rs713041) and SOD2 gene (rs4880) in the development of abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease (AOID), as well as their associations with cardiac diseases and function in patients were evaluated.

Materials and methods. A series of 516 AAA patients, 352 AOID patients, and 510 controls were analyzed. Patients were characterized in terms of coronary and non-coronary atherosclerosis and its complications. Genotyping was performed using the TaqMan-based assays. Selenoprotein P (SeP) and thioredoxin (Trx) levels in plasma were assessed by ELISA.

Results. Higher plasma SeP levels were associated with more favorable cardio-metabolic profile in our patients (BMI values, HDLC, TG levels). In AOID, as compared to AAA, the increased frequency of the GPX4 rs713041TT homozygotes was observed: OR=1.66, P=.023 (OR=1.62, P=.046 for AAA without concomitant peripheral atherosclerosis). In patients with systolic heart failure, lower frequency of carriers of the SEPP1 rs3877899G-rs7579G haplotype, and increased levels of studied selenoproteins were found. Higher plasma selenoprotein levels were also associated with presence of small AAA.

Conclusions. This study identified variant alleles of the selenoprotein genes as potential genetic markers that indicate predisposition to occlusive and aneurysmal types of arterial disease and to systolic heart failure. Expression of selenoproteins seems to be induced during AAA and heart failure development, to protect against oxidative stress. Supported by the NSC in Poland under NN403250440 grant, and PUMS under 502-01-02214335-05962 and 502-14-02214335-10268 grants.

#### P05.03

#### Association between C reactive protein and abdominal aortic aneurysm: Mendelian randomization analysis

*J. Juan<sup>1</sup>, X. Qin<sup>1</sup>, Y. Wei<sup>2</sup>, K. Sun<sup>1</sup>, S. Zuo<sup>3</sup>, W. Guo<sup>3</sup>, Y. Hu<sup>1</sup>;*

<sup>1</sup>Peking University, Beijing, China, <sup>2</sup>Beijing Center for Diseases Prevention and Control, Beijing, China, <sup>3</sup>Chinese PLA general hospital & medical school PLA, Beijing, China.

Introduction: Several studies have suggested C-reaction protein (CRP) is associated with the development of abdominal aortic aneurysm (AAA), but it is unclear whether this association is causal. To avoid confounding or reverse causality, we used Mendelian randomization analysis to study the causal role of CRP and diameter of abdominal aorta.

Materials and Methods: 465 Chinese participants were included in the analysis. CRP gene rs1205 was used as an instrumental variable of plasma high-sensitivity CRP level. We assessed the association of hs-CRP levels with diameter of abdominal aorta and other risk factors or confounders in the conventional models, and also assessed the associations of the gene with the diameter of abdominal aorta, hs-CRP and other factors. We used two-stage least squares for the Mendelian randomization analyses to assess the relationship between the expected hs-CRP level estimated from the gene and the diameter of AAA.

Results: In the conventional analysis, hs-CRP was associated with AAA ( $P<0.001$ , 95% CI 0.03-0.12). rs1205 in CRP gene was associated with hs-CRP levels ( $P<0.0001$ ), but unrelated to other factors, including age, gender, smoking, drinking and history of hypertension. The results of Mendelian randomization analysis showed a null association of hs-CRP with AAA

( $P=0.71$ , 95% CI -0.30-0.20). The first-stage F-statistics was 12.87, indicating sufficient strength to ensure the validity.

Conclusions: Mendelian randomization analysis indicates that hs-CRP level may not be causally associated with AAA in this population. This suggests that the results of conventional analysis may be affected by confounding or reverse causality.

#### P05.05

#### Is there any association between insulin resistance and apelin gene expression in non-diabetic subjects?

*M. Zarkesh<sup>1</sup>, E. Yuzbashiyan<sup>2</sup>, G. Asghari<sup>2</sup>, M. Hedayati<sup>1</sup>, P. Mirmiran<sup>2</sup>, A. Khalaj<sup>3</sup>;*

<sup>1</sup>Cellular and Molecular Endocrine Research Center, Research Institute for endocrine sciences Shaheed Beheshti University of Me, Tehran, Iran, Islamic Republic of, <sup>2</sup>Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of, <sup>3</sup>Obesity Treatment Center, Department of Surgery, Shahed University, Tehran, Iran, Tehran, Iran, Islamic Republic of.

**Background and aim:** Adipose tissue-derived hormones like apelin play a functional role in the glucose tolerance through its effects on insulin secretion and insulin sensitivity which is also a risk factor for type 2 diabetes. In the current study, we aimed to investigate the association of apelin mRNA expression in omental and subcutaneous adipose tissues with fasting glucose, insulin, and insulin resistance among non-diabetic subjects.

**Material & methods:** Under a cross-sectional analytical design a total of 62 non-diabetic subjects, aged  $\geq 18$  years, were eligible for the study. Omental and subcutaneous adipose tissues were obtained during open abdominal surgery. The mRNA expression of apelin in omental and subcutaneous adipose tissues were assessed by Real-Time PCR. Serum levels of apelin, triglycerides, total cholesterol, glucose, and insulin were measured and homeostatic model assessment of insulin resistance index (HOMA-IR),  $\beta$ -cell function (HOMA-B), and the quantitative insulin sensitivity check index (QUICKI) were calculated.

**Results:** Apelin mRNA expression in omental adipose tissue was positively correlated with insulin levels ( $r=0.281$ ,  $P=0.034$ ) and HOMA-IR ( $r=0.270$ ,  $P=0.043$ ) and negatively correlated with QUICKI ( $r=-0.270$ ,  $P=0.043$ ). In addition, apelin gene expression in subcutaneous adipose tissue was negatively correlated with triglycerides levels ( $r=-0.404$ ,  $P=0.041$ ).

**Conclusion:** In conclusion, our data indicate that up-regulation of apelin in omental adipose tissue is associated with glucose hemostasis and insulin resistance.

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#### P05.06

#### Clinical application of a multigene panel associated with many inherited cardiac conditions: challenges of defining risks, clinical outcomes and off-target findings

*D. du Sart<sup>1</sup>, B. Chong<sup>1</sup>, S. Pantaleo<sup>1</sup>, I. Macciocca<sup>1</sup>, P. James<sup>2</sup>;*

<sup>1</sup>Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Melbourne, Australia, <sup>2</sup>Clinical Genetics, Royal Melbourne Hospital, Melbourne, Australia.

Introduction: Genetic testing for inherited cardiac conditions impacts on clinical management options for families. These conditions have complex genetic pathology, often heterogeneous disorders with overlapping clinical symptoms that are not always penetrant. Classifying variants as pathogenic, as influencing clinical severity or just normal variation is complex.

Materials and Methods: Multigene panels are cost- and time-effective analyses to identify familial pathogenic variants. Patients with inherited arrhythmia, cardiomyopathy, aortopathy and sudden death conditions are screened with a 101-cardiac-gene NGS panel. Gene variants are analysed according to patient phenotype, but the analysis flags variants classified as pathogenic in all genes.

Results: More than 1000 probands were tested, followed by segregation analyses for clinically actionable variants (see table). Pathogenic variants in "off-target" genes (1.6%) lead to many challenges for both laboratory and clinical teams. Biological interpretation of variants suggest a strong association with clinical disease, but lack of information on clinical outcomes make pathogenic classification uncertain. Counselling challenges included conveying unexpected information about different cardiac genes. Clinical assessment revealed no evidence of clinical association with the "off-target" genotype.

Conclusion: Pathogenic variants in "off-target" genes identify significant gaps in our gene-specific phenotype knowledge base. Documentation of these cases will improve knowledge about the spectrum of disease associated with genes and will impact on future management in families with asso-

ciated phenotypes. Does this justify the complexities raised by "off-target" gene variants?

	Arrhyth-mia	Cardiomy-opathy	Aortopa-thy	Sudden Death / Extended Panel
Pathogenic / Likely pathogenic variant	45%	51%	26 %	33%
Variant of unknown significance	24%	38%	34%	57%
No clinically actionable variant	31%	11%	40%	10%
"Off-target" gene pathogenic variants in 1000 probands	10 probands: 7 stop/ frame shift 3 splice [+1]	2 probands -pathogenic in LQT	4 probands 3 stop 1 splice [-1]	

## P05.07

### Desmosomal genetic variants in young competitive athletes: pathogenic mutations or innocent bystanders?

*M. Cason<sup>1</sup>, M. Schiavon<sup>2</sup>, A. Zorzì<sup>1</sup>, R. Celeggin<sup>1</sup>, E. Lazzarini<sup>1</sup>, E. Carturan<sup>1</sup>, B. Bause<sup>1</sup>, I. Rigato<sup>1</sup>, C. Basso<sup>1</sup>, D. Corrado<sup>1</sup>, G. Thiene<sup>1</sup>, K. Pilichou<sup>1</sup>;*

<sup>1</sup>Dept. of Cardiac, Thoracic and Vascular Sciences, University of Padua, Padova, Italy,

<sup>2</sup>Sports Medicine & Phys. Activities Unit, ULSS 16, Padova, Italy.

**Introduction.** Arrhythmogenic Cardiomyopathy (AC) is an inherited myocardial disease characterized by fibro-fatty replacement of myocardium and increased risk of sudden death (SD), which may be the first disease manifestation in young and athletes. About half of AC patients harbor genetic variants in genes encoding for desmosomal proteins.

**Purpose.** To assess the frequency of rare desmosomal variants in a cohort of apparently healthy athletes in order to evaluate their pathogenic role.

**Methods.** 188 unrelated young athletes (mean age 20 yrs, male/female ratio 3:1), eligible at the pre-participation clinical evaluation, underwent conventional genetic screening for major disease causative genes: Desmoglein2-DSG2, Desmoplakin-DSP, Plakophilin2-PKP2, Desmocollin2-DSC2, Plakoglobin-JUP. Variants selection was based on the absence or low frequency (minor allele frequency <0.0002) of the genetic variants in the general population, aminoacid conservation across species, in silico pathogenic evaluation.

**Results.** Genetic screening identified putative pathogenic rare genetic variants in 8 (4.26%) athletes: 3 in DSG2, 2 in DSP and PKP2 respectively and 1 in DSC2. Only one of these variants was also reported in a patient with definite AC criteria. All 8 genotype positive athletes had no family history for AC or SD and showed no clinical signs of the disease at the second-level cardiological evaluation.

**Conclusion.** Our study demonstrated the presence of rare genetic desmosomal variants in healthy athletes, questioning their contribution in AC phenotype. The presence of desmosomal variants in the healthy population highlights the importance of clinical evaluation and cascade genetic screening in the families of mutation carriers to determine genetic variants pathogenicity.

## P05.08

### Contribution of non desmosomal variants in AC pathogenesis.

*R. Celeggin, E. Lazzarini, I. Rigato, M. Cason, E. Carturan, D. Corrado, G. Thiene, B. Bause, C. Basso, K. Pilichou;*

*University of Padua, Padova, Italy.*

**Introduction.** Arrhythmogenic Cardiomyopathy (AC) is an inherited myocardial disease characterized by fibro-fatty replacement of myocardium, mainly linked to mutations in desmosomal genes. Next Generation Sequencing (NGS) enables the analysis of many genes at the same time in a single run. **Purpose.** We developed a targeted NGS panel for the parallel analysis of 150 genes associated with different cardiomyopathies in the diagnostic setting. **Materials and methods.** 62 AC patients were analyzed using NGS technology on a MiSeq platform (Illumina) using a custom panel of 150 genes associated with cardiomyopathies. The selection of the detected variants was based on the frequency, the conservation rate of the aminoacid, the potential pathogenicity and the functional effect on the protein.

**Results.** We identified 92 potentially pathogenic variants in desmosomal genes in 27 patients (43%), 7 of them carried 11 additional non-desmosomal variants, while 26 probands (44%) carried exclusively rare variants in sarcomeric (MYO6, MYH7, MYBPC3, MYH13, TNNI3, TNNT2) and ion-channel genes (KCNQ3, CACNA1C, SCNN1G, TRPM4, SCN5A, SCN10A, KCNA5,

KCNE3, CACNA1B, KCNQ1, KCNK3).

**Conclusions.** The presence of rare likely pathogenic variants in non-desmosomal genes in AC probands may exert independent or synergistic effect on pathogenesis of AC. Deeper investigation is necessary to determine the role of these variants on the onset of the clinical phenotype. Cascade genetic screening remains imperative to clarify their role in AC families and to identify relatives at risk.

## P05.09

### Mutation screening of a panel of genes involved in inherited cardiomyopathies by using targeted next generation sequencing

*M. Calore<sup>1,2</sup>, I. E. A. Li Mura<sup>3</sup>, A. Telatin<sup>3</sup>, I. Zara<sup>3</sup>, A. Lorenzon<sup>2</sup>, M. De Bortoli<sup>2</sup>, G. Poloni<sup>2</sup>, B. Bause<sup>4</sup>, B. Simonati<sup>3</sup>, A. Rampazzo<sup>2</sup>;*

<sup>1</sup>Department of Cardiology, School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, <sup>2</sup>Department of Biology, University of Padua, Padua, Italy, <sup>3</sup>BMR Genomics, Padua, Italy, <sup>4</sup>Department of Cardiac, Thoracic, and Vascular Sciences, University of Padua, Padua, Italy.

Inherited cardiomyopathies are caused by mutations in different genes, which makes genetic screening particularly challenging. Targeted-next-generation sequencing (NGS) enables mutation detection in large cohorts of patients affected with genetically heterogeneous diseases.

**Purpose:** developing and validating a targeted-NGS system for the genetic diagnosis of inherited cardiomyopathies.

**Methods:** Mutation screening was performed in 19 probands affected with arrhythmogenic cardiomyopathy (ACM), 2 with hypertrophic cardiomyopathy, and 1 with dilated cardiomyopathy, using a NGS gene-panel comprising the exons and adjacent boundaries of 56 genes involved in the main inherited cardiomyopathies. Target capture was performed by HaloPlex kit (Agilent) and samples were sequenced on a MiSeq sequencer (Illumina) at BMR-Genomics (Padua). Variants were called using GATK Unified Genotyper, GATK Haplotype Caller, FreeBayes and VarScan2 and annotated with Annovar and SNP-Shot (BMR-Genomics), the latter used also for variant prioritization.

**Results:** We identified 38 variants validated by Sanger sequencing (SS) and 2 expected false positives (unbalanced variants) and we confirmed 25 out of 27 variants previously detected by SS. Coverage >20X was obtained for 91.95% of 1.416.998 analyzed nucleotides and 90.93% of ACM genes. Among ACM patients, 11 carried mutations in ACM genes and 3 genotype-negative carried variants in genes associated to other cardiomyopathies.

**Conclusions:** Our approach represents a valuable tool for mutation screening in patients affected with inherited cardiomyopathies. Optimization of target capture will further improve the ability of mutation detection.

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## P05.10

### Diagnostic utility of the extended next generation sequencing gene panel in a cohort of 31 Czech patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C): a pilot study

*L. Piherová<sup>1</sup>, J. Paderová<sup>2</sup>, A. Krebsová<sup>3</sup>, P. Kubus<sup>4</sup>, M. Kubanek<sup>3</sup>, J. Kautzner<sup>3</sup>, J. Janousek<sup>4</sup>, S. Kmoch<sup>1</sup>, M. Macek Sr.<sup>2</sup>, M. Macek Jr.<sup>2</sup>;*

<sup>1</sup>Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine and VFN, Prague, Czech Republic, <sup>2</sup>Department of Biology and Medical Genetics, 2nd Faculty of Medicine and FN Motol, Prague, Czech Republic, <sup>3</sup>Department of Cardiology, Institute of Clinical and Experimental Medicine, Prague, Czech Republic, <sup>4</sup>Children's Heart Centre, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.

**Introduction:** ARVD/C is a rare hereditary cardiovascular disorder associated with a higher risk of sudden cardiac death.

**Patients and Methods:** Altogether, 31 cases with clinical diagnosis of ARVD/C underwent genetic counseling followed by TruSight One panel sequencing (Illumina, USA). Detected variants, classified according to ACMG.net, were confirmed by Sanger DNA sequencing and familial segregation analysis, where possible.

**Results:** Positive family history was present only in 3 cases (10%) with severe ARVD/C. Likely pathogenic variants were revealed in 22/31 (71%) of patients in known ARVD/C-associated genes, mainly PKP2 in 10/31 of all cases (32%). In addition, variants in DSP, DSG, DSC were identified in 2/31 (6% each). Likely pathogenic variants in DES, TMEM43, TGFBI3, ANK3, LDB3, JPH2 and SCN5A occurred once (3% each). One compound heterozygote for DSP/JPH2 mutations was revealed. In 9/31 patients (29%) the molecular etiology was not elucidated by our approach. Only 4/9 patients did not have a detectable DNA variant, in 5/9 patients variants in genes associated with other type of hereditary cardiovascular disease were observed (e.g. KCNE3/

TRPM4, SCN10A/TRDN, TRPM2, SOS1, FBN1).

Conclusion: We carried out a pilot study of ARVD/C variant distribution by an extended next generation sequencing panel in a representative cohort of Czech patients with a predominantly severe ARVD/C. Majority of likely pathogenic variants were found in PKP2 and the significance of DNA variants in other "non-ARVD/C-related" genes should be further assessed in order to provide clinical guidance and risk assessment in extended families.

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#### P05.11

##### Association study of polymorphic variants in leading candidate-genes from genome-wide association study in Bulgarian coronary artery disease patients and healthy population controls

R. Tzveova<sup>1</sup>, G. Naydenova<sup>2</sup>, T. Yaneva-Sirakova<sup>3</sup>, G. Dimitrov<sup>3</sup>, S. Vandeva<sup>4</sup>, D. Pendicheva<sup>5</sup>, P. Atanasov<sup>6</sup>, I. Popov<sup>7</sup>, C. Naydenov<sup>8</sup>, R. Turnovska-Kadreva<sup>9</sup>, G. Nachev<sup>9</sup>, V. Mitev<sup>1</sup>, R. Kaneva<sup>1</sup>;

<sup>1</sup>Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, <sup>2</sup>Second Department of Cardiology, UMHAT „Dr. Georgi Stranski“, Pleven, Bulgaria, <sup>3</sup>Department of Internal Medicine, UMHAT „Alexandrovska“, Cardiology clinic, Medical University, Sofia, Bulgaria, <sup>4</sup>Clinical Center of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria, <sup>5</sup>Department of Experimental and Clinical Pharmacology, Medical University, Pleven, Bulgaria, <sup>6</sup>National Sport Academy, Sofia, Bulgaria, <sup>7</sup>Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, <sup>8</sup>University National Specialized Hospital for Active Treatment Hospital "Sv. Ekaterina", Medical University, Sofia, Bulgaria.

The polymorphic variants in or near NRP3, ATP2B1 and CDH13 genes have been found to be associated with blood pressure measurements in some European populations. Hypertension is a major cause for coronary atherosclerosis, therefore the aim of this study was to investigate the possible association of three markers, rs1173771 (NRP3-C5orf23), rs2681472 (ATP2B1), and rs11646213 (CDH13), with the risk of coronary artery disease (CAD) and myocardial infarction (MI) in Bulgarians.

The study included both 324 CAD patients (199 with MI and 125 without MI) and 496 population controls. The studied variants were genotyped by using TaqMan genotyping assays. Genotype and allele frequencies were calculated and associations with disease were estimated using  $\chi^2$ .

The polymorphic variant rs1173771 did not show association with coronary atherosclerosis in the studied group (Allele OR1.03;CI0.78-1.37;p=0.58). The G allele of rs2681472 showed a link to higher risk of both CAD without MI(OR1.40;CI1.09-1.82;p=0.01) and with MI (OR1.48;CI0.10-1.99;p=0.009). In the subgroup analysis, this dependence was recorded only in men with CAD without MI(OR1.60;CI1.14-2.24;p=0.01). Furthermore, the same allele was associated with an increased risk of MI in men (OR1.87;CI1.29-2.72;p=0.001). Finally, allele A of rs11646213 showed an association with lower risk of MI(OR0.56;CI 95:0.38-0.85;p=0.005).

In this study we found for the first time association between investigated polymorphisms and CAD with/without MI in Bulgarians. Further studies in larger cohorts are needed to confirm the obtained results.

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#### P05.13

##### Barth syndrome phenotypically manifested in a heterozygous female carrier

T. Todorov<sup>1</sup>, D. Avdjieva-Tzavella<sup>2</sup>, H. Kathom<sup>2</sup>, I. Yordanova<sup>1</sup>, M. Ivanova<sup>3</sup>, R. Tincheva<sup>2</sup>, A. Todorova<sup>4,1</sup>;

<sup>1</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria, <sup>2</sup>University Pediatric Hospital, Medical University Sofia, Sofia, Bulgaria, <sup>3</sup>National Genetic Laboratory, University Hospital " Majcín Dom ", Sofia, Bulgaria, <sup>4</sup>Medical University Sofia, Sofia, Bulgaria.

Barth syndrome (BTHS) is an X-linked recessive disease typically characterized by dilated cardiomyopathy, predominantly proximal skeletal myopathy, prepubertal short stature and neutropenia. From biochemical point of view the disease is associated with excess of 3-methylglutaconic acid, hypoglycemia, lactic acidosis, hyperammonemia. The disease is caused by mutations in the tafazzin gene (TAZ) which lead to cardiolipin deficiency and mitochondrial dysfunction. Male patients have variable clinical findings, while female carriers are usually asymptomatic.

The TAZ gene was screened for mutations by Sanger sequencing (using Big-Dye terminator v3.1 Cycle Sequencing Kit) in male and female siblings both with left ventricular noncompaction and hypotonia. Additionally, the brother presented an intermittent

neutropenia and increased urinary levels of 3-methylglutaconic and 3-methylglutaric acid. The molecular genetic testing of the TAZ gene revealed a novel mutation c.253insC, p.(Arg85Profs\*54) in exon 3 of the TAZ gene. Both siblings carry the same mutation. The mutation was inherited from asymptomatic mother.

To the best of our knowledge this is the first case of Barth syndrome phenotypically manifested in a heterozygous female carrier with normal karyotype. A possible explanation could be non-random X inactivation predominantly involving the non-affected X chromosome.

#### P05.14

##### Exome-wide association study fails to identify genetic variation contributing to the development of bicuspid aortic valve

M. Gago-Díaz<sup>1,2</sup>, C. Newton-Cheh<sup>3,4</sup>, S. Body<sup>4,5</sup>, Á. Carracedo<sup>2,6</sup>, G. Teixidó-Turà<sup>7</sup>, D. García-Doradó<sup>7</sup>, A. Evangelista<sup>7</sup>, B. María<sup>1,2</sup>;

<sup>1</sup>Xenética de enfermedades cardiovasculares e oftalmológicas, Instituto de Investigación Sanitaria de Santiago de Compostela, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, <sup>2</sup>Grupo de Medicina Xenómica, Instituto de Investigación Sanitaria de Santiago de Compostela-Universidade de Santiago de Compostela-Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain, <sup>3</sup>Cardiovascular Research Center and Center for Human Genetic Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, <sup>4</sup>The Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, United States, <sup>5</sup>Anesthesia and Pain Management, Brigham and Women's Hospital, Boston, MA, United States, <sup>6</sup>Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia, <sup>7</sup>Servei de Cardiología, Hospital Universitari Vall d'Hebron, Barcelona, Spain.

Introduction: Bicuspid aortic valve is the most common cardiovascular congenital malformation affecting humans with a prevalence of 0.5- 2% in general population. Although it can be diagnosed as an incidental finding, many patients develop life-threatening complications that have awoken interest in understanding a molecular basis that remains unknown. Based on 90% heritability and high incidence of familial clustering, our objective was to identify any genetic factor that could predict patients at risk of developing symptomatology.

Materials and Methods: Assuming a complex mode of inheritance, we conducted a transversal exome-wide association study in a discovery cohort of 570 cases and 484 Spanish controls that we genotyped with the Axiom Exome Array (Affymetrix) and imputed association results based on the Phase 3 1000 Genomes Project reference panel. We performed association analysis for the total sample and 6 different subgroups classified based on thoracic aortic measurements and valve morphology. The replication series comprised 512 cases and 1,483 European controls that we genotyped with MassArray System (Sequenom). Finally, we performed meta-analysis using METAL.

Results: We prioritized 13 markers identified during the discovery study for replication along with 5 FBN1 markers previously described as associated, but were not able to replicate any result.

Discussion and Conclusions: These negative results support bicuspid aortic valve complexity (that should not longer be considered a unique entity), the need to establish worldwide collaborations to increase sample size and power and the association of FBN1 with thoracic aortic dilation and not the valvular malformation itself. Funding: PI13/00933 and RD12/0042/0037, ISCIII-FEDER.

#### P05.15

##### Genetic characterization of a BAV population of Italian origin. Results from GISSI OUTLIERS VAR study.

S. Pileggi<sup>1</sup>, S. Barlera<sup>1</sup>, B. Merlanti<sup>2</sup>, B. De Chiara<sup>2</sup>, L. Martinelli<sup>3</sup>, A. Maseri<sup>4</sup>, M. G. Franzosi<sup>1</sup>;

<sup>1</sup>Department of Cardiovascular Research, IRCCS Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy, <sup>2</sup>Department of Cardiology and Cardiovascular Surgery, Niguarda Ca' Granda Hospital, Milan, Italy, <sup>3</sup>Cardiothoracic Surgery, ICLAS-Istituto Clinico Ligure Alta Specialità, Rapallo, Italy, <sup>4</sup>Heart Care Foundation, Florence, Italy.

Introduction: Bicuspid aortic valve (BAV) formation is genetically determined, with reduced penetrance and variable expressivity. NOTCH1 gene is a well-proved candidate gene and its mutations have been found both in familial and sporadic cases of BAV.

Materials and Methods: 66 BAV patients from the GISSI VAR study have been genotyped for the NOTCH1 gene.

Results: We identified 64 variants, both in heterozygous and in homozygous state. Fifty-two are common polymorphisms present in almost all patients. Eleven variants are new and never reported in literature: two are non-synonymous substitution, Gly540Asp in exon 10 and Glu851Gln in exon 16;

one is in the 3'UTR region and 7 in introns, one corresponds in a T allele insertion in intron 27. We selected four statistically relevant and seven new variants identified in six BAV patients and correlated them with clinical and demographic variables as well as imaging and histological parameters. Our preliminary data show that 4 were BAV patients with isolated stenosis in over 60 year-old patients. We suppose that these variants correlate with a late necessity to undergo to surgery that is driven by the presence of stenosis and not by aortic valve regurgitation or ascending aortic aneurysm. Conclusions: We completed the genotyping of 66 BAV patients and found 11 new variants in the NOTCH1 gene never reported in literature. These data confirm that the identification of new clinically relevant biomarkers for BAV requires a deeper genetic understanding of the NOTCH1 gene variants, that could be targeted by future diagnostic and therapeutic strategies.

#### P05.16

#### Copy number variation in clinical tests for inherited cardiomyopathies and arrhythmias

*J. Tahiliani, N. Johnson, S. Aguilar, A. Daly, E. Decker, B. Herrera, D. Beltran, L. Murillo, R. Harte, J. Garcia, S. Lincoln, E. Haverfield, S. Aradhya; Invitae, San Francisco, CA, United States.*

**Introduction:** The clinical significance of exon-level copy number variation (CNV) in inherited cardiovascular conditions has not been widely studied to date. Sequence changes make up the vast majority of reported pathogenic variants in genes associated with inherited cardiomyopathies and arrhythmias.

**Methods:** We considered a sequential series of 287 patients submitted for an arrhythmia panel, 370 for a cardiomyopathy panel, and 36 for both. Germline DNA from blood was tested, and the gene panels were selected by ordering physicians based on each patient's clinical indication. Sequence and CNV analysis was performed by validated Next Generation sequencing (NGS) methods, and identified CNVs were confirmed by microarray analysis. **Results:** Six single- or multi-exon deletions were identified in patient samples: two deletions in MYBPC3 in two unrelated patients presenting with hypertrophic cardiomyopathy; two RYR2 deletions in two unrelated patients presenting with arrhythmia; a PKP2 deletion in a patient presenting with arrhythmogenic cardiomyopathy; and a CTNNA3 deletion in a patient presenting with cardiomyopathy. Except CTNNA3, all deletions were classified as pathogenic, and no other pathogenic variants were identified in these individuals.

**Conclusions:** Historically, CNV analysis for these genes was either not widely available clinically or was available as a reflex test. In this case series, CNVs accounted for about 4% of the positive findings, suggesting that CNV analysis may be an important component of genetic testing for inherited cardiomyopathies and arrhythmias in the future.

#### P05.17

#### Genetic screening in idiopathic cardiomyopathy: should we broaden our perspective?

*E. Vanhoutte<sup>1</sup>, M. Hazebroek<sup>2</sup>, L. Montfort<sup>1</sup>, M. Witjens<sup>1</sup>, M. Hoos<sup>2</sup>, A. Helderma-van den Enden<sup>1</sup>, F. van Tienen<sup>1</sup>, G. Claes<sup>1</sup>, A. Wijngaard<sup>1</sup>, S. Heymans<sup>2</sup>, I. Krapels<sup>1</sup>;*

<sup>1</sup>*Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, Netherlands, <sup>2</sup>Department of Cardiology, Maastricht University Medical Center, Maastricht, Netherlands.*

**Introduction:** The ESC guidelines recommend genetic testing merely in patients with dilated cardiomyopathy (DCM). However, a significant subgroup presents with left ventricular (LV) dysfunction without dilatation. Our aim is to compare the yield of NGS gene testing and prognosis in LV dysfunction patients versus DCM patients.

**Materials and Methods:** 262 DCM and 127 LV dysfunction patients underwent complete cardiologic evaluation and NGS gene testing of 46 cardiomyopathy genes. Genetic or familial predisposition was defined as familial inheritance pattern and/or a (likely) pathogenic mutation. Long-term outcome used a combined endpoint of life-threatening arrhythmia, heart transplantation, and death.

**Results:** A genetic or familial predisposition was equally distributed between DCM and LV dysfunction patients (100 (38%) vs 57 (45%);  $P=0.21$ ). A (likely) pathogenic mutation was more frequently found in patients with positive familial inheritance, irrespective of a DCM or LV dysfunction phenotype (20 (25%) vs 17 (9%);  $P=0.001$  and 12 (27%) vs 11 (13%)  $P=0.049$ , respectively). After a median follow-up of 56 months, long-term outcome did not differ between DCM and LV dysfunction. However, in both DCM and LV dysfunction patients outcome was worse in the presence of a genetic/familial predisposition.

**Conclusion:** The diagnostic yield of NGS gene testing is comparable between

DCM and LV dysfunction without dilatation. Prognosis is worse in both DCM and LV dysfunction patients with a genetic or familial predisposition. Therefore, genetic screening should also be performed in patients with LV dysfunction.

#### P05.18

#### Genetic testing in dilated cardiomyopathies: increasing the diagnostic yield by next generation sequencing techniques

*A. T. J. M. Helderma-vd Enden<sup>1</sup>, M. Riemersma<sup>1</sup>, E. K. Vanhoutte<sup>1</sup>, L. van Montfort<sup>1</sup>, M. I. Witjens<sup>1</sup>, M. B. Hoos<sup>2</sup>, M. R. Hazebroek<sup>2</sup>, F. H. J. van Tienen<sup>1</sup>, G. F. R. Claes<sup>1</sup>, S. R. B. Heymans<sup>2</sup>, I. P. C. Krapels<sup>1</sup>, A. van den Wijngaard<sup>1</sup>;*

<sup>1</sup>*Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, Netherlands, <sup>2</sup>Department of Cardiology, Maastricht University Medical Center, Maastricht, Netherlands.*

**Purpose:** Dilated cardiomyopathy (DCM) is characterized by ventricular chamber enlargement and systolic dysfunction. Nowadays 30-50 genes are associated with DCM and a genetic cause of this heterogeneous disorder is found in 30-40% of all patients. The aim of this study was to increase the diagnostic yield of genetic testing by using next-generation sequencing (NGS) techniques.

**Methods:** In 406 idiopathic DCM patients Sanger sequencing was performed for several genes. A subgroup of 172 patients received one or both of the following gene panel tests: CardioChip (34 genes) and next-generation whole exome sequencing using a targeted filter (45 genes). In the gene panel subgroup, *TTN* was tested in 93 patients. Genetic variants were defined as variants of unknown clinical significance (VUS), likely pathogenic (LP) and pathogenic (P) variants.

**Results:** Sanger sequencing of several genes detected a genetic variant in 16.5%. Genetic variants were found in 46% by gene panel testing. In the gene panel subgroup of 172 patients, a variant in *MYH7*, *PLN* and *LMNA* was detected in 4.1%. A variant in other genes was found in 40.7%. The highest detection rates of likely pathogenic variants or pathogenic variants were found in *TTN* (13%), *RBM20* (3.2%) and *MYH7* (2.5%). **Conclusions:** Our data illustrate that gene panel testing in DCM patients increases the diagnostic yield, with the highest diagnostic yield in *TTN*. We suggest that genetic analysis in idiopathic DCM patients starts with NGS gene panel testing without prior single gene testing.

#### P05.19

#### Detection of known and novel cardiovascular diseases gene variants by next generation sequencing in terms of improved treatment and prognosis

*S. Josifovska<sup>1,2,3</sup>, R. Vazharova<sup>1,4</sup>, L. Balabanski<sup>1</sup>, S. Ivanov<sup>1</sup>, R. Staneva<sup>2</sup>, S. Hadjidekova<sup>2</sup>, M. Malinov<sup>1</sup>, S. Panov<sup>2</sup>, D. Toncheva<sup>2</sup>;*

<sup>1</sup>*SBALGAR "Malinov", Sofia, Bulgaria, <sup>2</sup>Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria, <sup>3</sup>Faculty of Natural Sciences and Mathematics, "Ss. Cyril and Methodius" University, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>4</sup>Medical Faculty, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria.*

**Introduction:** According to the WHO, cardiovascular diseases (CVD) are the main cause of death worldwide. CVD involve a large group of heterogeneous disorders that cannot always be precisely diagnosed, so therapy and outcome heavily depend on proper genetic profiling. The aim of this study was to determine genetic variants in patients with CVD.

**Materials and Methods:** DNAs from 11 patients with CVD were analyzed using NGS targeted resequencing of 176 genes included in TruSight Cardio gene panel (Illumina). Variant detection was upon reference human genome (GRCh37).

**Results:** Detected gene variants are as follows: heterozygous *ACTA2*:p.Arg258Cys, in a patient with aortic dissection; heterozygous *KCNJ2*:p.Arg218Trp in a patient with LQTS; double heterozygosity for *CACNA1C*:p.Arg514Gly and *SCN5A*:p.Arg800His in a patient with severe hypertrophic obstructive cardiomyopathy and arrhythmia; double heterozygosity for *ELN*:c.890-1G>A and *SCN5A*: p.Gly9Val in a patient with SVAS, pulmonary valve stenosis and arrhythmia. An interesting finding is the co-occurrence of dominant pathogenic variants in different genes, as in the case of two of the patients, which may explain the complexity and severity of the observed phenotypes.

**In conclusion:** detection of common and new variants in the patients with CVD has confirmed or shifted diagnosis, further establishing genetic association studies involving NGS as analysis of choice for clinical diagnosis and research with large implication on treatment and prognosis of patients and their families.

## P05.21

**Molecular autopsy: a potential tool to identify pathogenic CNVs in congenital heart defects**

F. A. R. *Madia*<sup>1</sup>, C. *Milani*<sup>1</sup>, A. T. *Dias*<sup>1</sup>, E. A. *Zanardo*<sup>1</sup>, J. G. *Damasceno*<sup>1</sup>, M. *Rocha*<sup>2</sup>, A. M. *Nascimento*<sup>1</sup>, T. V. M. M. *Costa*<sup>1</sup>, G. M. *Novo-Filho*<sup>1</sup>, M. M. *Montenegro*<sup>1</sup>, A. F. *Amorim*<sup>1</sup>, Y. G. *Oliveira*<sup>1</sup>, C. H. *Chung*<sup>1</sup>, J. R. M. *Ceroni*<sup>1</sup>, R. *Schultz*<sup>1</sup>, F. T. *Gonçalves*<sup>1</sup>, C. *Fridman*<sup>1</sup>, C. A. *KIM*<sup>1</sup>, L. D. *Kulikowski*<sup>1,2</sup>;

<sup>1</sup>Faculdade de Medicina da USP, SAO PAULO, Brazil, <sup>2</sup>Faculdade de Medicina do ABC, Santo André, Brazil.

**Introduction:** Congenital heart defects (CHD) is the most prevalent group of congenital malformations affecting approximately 8-1,000 live births and is a significant cause of childhood morbidity and mortality. Recently, the increased frequency of pathogenic copy number variants (CNVs) in congenital heart disease was demonstrated by several different studies.

**Materials and Methods:** We investigate the contribution of CNVs in the pathogenesis of CHD using molecular methods in 29 cases of stillbirth and new-born from Serviço de Verificação de óbitos, HC-FMUSP. DNA samples from skin and heart tissues were evaluated using AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Life Technologies™, California, USA) and Multiplex Ligation-dependent Probe Amplification (MLPA) with different kits (MCR-Holland, Amsterdam, the Netherlands).

**Results:** The results showing several different CNVs such as ZNF74, NCAM2, NFATC1 and AR genes associate with cardiac phenotype. In 9 of 28 stillbirth and new-born (32%) with CHD and additional structural abnormalities, we identified cases of Edwards Syndrome, Patau Syndrome, Down Syndrome and one case of Phelan-McDermid Syndrome with duplication of ZNF74. Also we identified one case of Turner Syndrome and one case of Turner Syndrome with Y chromosome mosaicism. Only one isolated CHD case did not showed pathogenic CNVs.

**Conclusion:** Molecular autopsy showed efficiency for identifying pathogenic CNVs associated with CHD and also allowed the adequate genetic counseling.

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## P05.22

**The novel coronary artery disease risk gene KIAA1462 regulates endothelial cell function**

P. D. *Jones*<sup>1</sup>, R. *Rajmohan*<sup>1</sup>, T. R. *Webb*<sup>1</sup>, N. J. *Samani*<sup>1,2</sup>;

<sup>1</sup>University of Leicester, Leicester, United Kingdom, <sup>2</sup>NIHR Leicester Cardiovascular Biomedical Research Unit, Leicester, United Kingdom.

Genome wide association studies have identified 56 chromosomal loci that are associated with coronary artery disease (CAD). However, for most of these loci both the gene responsible and the mechanism by which they affect CAD risk is unclear. The CAD-associated locus at 10p11.21 contains 9 CAD-associated SNPs, located within the gene *KIAA1462*, which encodes a poorly characterised cell junction protein. As the role of *KIAA1462* is poorly understood, we set out to assess its functional role in endothelial cells, using siRNA gene knock-down. Compared with a control siRNA, an siRNA directed at *KIAA1462* resulted in a consistent 80 % reduction in *KIAA1462* expression. *KIAA1462* knock down resulted in slower cell proliferation, with approximately 15% fewer cells after 48 hours of proliferation compared to control siRNA transfected cells (t-test after 48 hours, p=0.001). An *in vitro* wound healing assay showed approximately 30% slower migration in knock-down cells (p=0.004). We also found increased apoptosis in *KIAA1462* knock-down cells, with a 15% increase in apoptosis as measured by Caspase 3/7 activity (p=0.030). In addition, we used a matrigel tube-formation assay as an *in vitro* assay for angiogenic capability of the cells. This showed approximately a 32% reduction in total tube length formed in *KIAA1462* knock-down cells compared to controls (p=0.002). These data suggest that *KIAA1462* is involved in the regulation of endothelial cell function and angiogenesis and this may explain its involvement in CAD.

## P05.23

**Genotype based pathway analysis of genetic loci associated with coronary artery disease**

S. E. *Hamby*<sup>1,2</sup>, T. *Webb*<sup>1,2</sup>, N. J. *Samani*<sup>1,2</sup>;

<sup>1</sup>University of Leicester Cardiovascular Research Center, Leicester, United Kingdom,

<sup>2</sup>NIHR Leicester Cardiovascular Biomedical Research Unit, Leicester, United Kingdom.

**Introduction:** Genome wide association studies have identified 56 genetic loci associated with increased risk of coronary artery disease (CAD). Many contain genes not previously linked to disease and potentially involve hitherto unrecognised biological processes. Investigating differential expression of pathways between risk/non-risk genotypes may allow identification of novel molecular mechanisms.

**Materials and methods:** We analysed gene expression data from 849 Monocyte, and 684 Macrophage samples collected from 459 individuals with CAD/MI and 458 controls as part of the Cardiogenics transcriptomic study (Rotival M, et al. *PLOS Genet.* 2011;12:e1002367). We utilised the QuSAGE R package (Yaari G et al. *Nucleic Acids Res.* 2013;41(18):e170) to explore differential expression of pathways between risk/non-risk genotypes for CAD loci. QuSAGE is a pathway analysis tool, modelling overall pathway fold change between sample groups utilising probability density functions.

**Results:** For seven CAD loci, we identified genotype related differential expression in specific pathways at FDR q value <0.1, with functions encompassing cytokine signalling, inflammation, calcium homeostasis and muscle contraction (Table). For the 9p21 locus we observed distinct pathways in monocytes/macrophages suggesting cell type specific effects. Pathways differentially expressed at specific CAD loci in monocytes/macrophages

Locus Name	Cell type	Reactome Pathway Name	Log Fold Change	FDR
6p21.31	Macrophage	Synthesis of PIPs at the Golgi Membrane	-0.053	0.031
		Synthesis and Interconversion of Nucleotide Di and Triphosphates	-0.071	0.032
6p24.1	Macrophage	Metabolism of Nucleotides	-0.040	0.043
		Growth Hormone Receptor Signalling	-0.050	0.079
		GT Phase	0.033	0.079
		Elevation of Cytosolic Ca <sup>2+</sup> Levels	-0.091	0.079
		Striated Muscle Contraction	0.047	0.079
		Muscle Contraction	0.040	0.079
		IL 6 Signalling	-0.058	0.079
		Antigen Activates B Cell Receptor Leading to Generation of Second Messengers	-0.029	0.096
9p21	Monocyte	Peptide Ligand Binding Receptors	0.023	<0.1x10 <sup>-15</sup>
		Class A1 Rhodopsin Like Receptors	0.015	<0.1x10 <sup>-15</sup>
9p21	Macrophage	Innate Immune System	-0.011	<0.1x10 <sup>-15</sup>
		Cytokine Signalling in Immune System	-0.0084	<0.1x10 <sup>-15</sup>
11q22.3	Monocyte	Regulation of RHEB GTPase Activity by AMPK	-0.037	0.019
15q26.1	Monocyte	Immune System	0.0044	<0.1x10 <sup>-15</sup>
17q21.32	Monocyte	VEGF Ligand Receptor Interactions	-0.056	0.095

**Conclusions:** Using QuSAGE we have identified several pathways related to CAD associated variants. This methodology provides a useful tool to examine the biological mechanisms of complex disease genetics.

## P05.24

**Expression of COX7A1 as a potential marker of congenital heart defects**

A. *Dobosz*, M. *Bik-Multanowski*;

*Jagiellonian University Medical College, Krakow, Poland.*

\* Congenital heart defects occur in approximately 50% of children with Down syndrome and in 1% of general population. The most common defects include ventricular septal defect (VSD), common atrioventricular channel (CAVC) and atrial septal defect (ASD). These defects are usually detected by means of fetal sonographic examination performed during pregnancy. We aimed at defining a molecular blood marker, which could allow for early detection of such defects in case of prenatal testing, performed due to suspected congenital malformation.

\* Methods: \* We conducted a whole genome expression analysis in 21 children with Down syndrome, with or without congenital heart defect. We used microarray technology to compare the whole genome expression in blood mononuclears between both subgroups. Next we used GeneSpring software for data analysis. Moderated t-test was applied with the Westfall-Young correction for multiple comparisons to detect significant differences between compared groups.

\* Results: \* We found upregulation of the *COX7A1* gene in patients born with heart defect (Fold Change 2.83). The *COX7A1* protein is one of the nuclear-coded polypeptide chains of cytochrome c oxidase, the terminal oxidase in mitochondrial electron transport in the muscle cell, and can play a role in heart muscle pathology. Our results show potential value of assessment of *COX7A1* expression in blood as a screening test for prenatal detection of congenital heart defects.

The study was sponsored by the Polish National Science Centre (DEC-2011/03/B/NZ5/01328).

## P05.25

**The characterisation of a novel nonsense mutation in RYR2 using human induced pluripotent stem cells**

*C. Bailey<sup>1</sup>, A. Tijzen<sup>2</sup>, L. Maizels<sup>2</sup>, I. Huber<sup>2</sup>, G. Arbel<sup>2</sup>, A. Gepstein<sup>2</sup>, N. Moreton<sup>3</sup>, B. Brown<sup>4</sup>, L. Venetucci<sup>1</sup>, W. G. Newman<sup>1,3</sup>, L. Gepstein<sup>2</sup>;*

<sup>1</sup>*University of Manchester, Manchester, United Kingdom, <sup>2</sup>Technion - Israel Institute of Technology, Haifa, Israel, <sup>3</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom, <sup>4</sup>University Hospital of South Manchester, Manchester, United Kingdom.*

**Introduction:** Heterozygous missense mutations in RYR2 are associated with catecholaminergic polymorphic ventricular tachycardia. We identified a novel nonsense mutation p.(Arg4790\*) in RYR2 in a young woman following resuscitation from a cardiac arrest. We generated human induced pluripotent stem cells (hiPSCs) to characterise this mutation.

**Methods:** We generated hiPSCs from the affected individual and differentiated these cells into cardiomyocytes (hiPSC-CMs). We performed confocal calcium imaging on the hiPSC-CMs and undertook analysis to determine the molecular mechanism by which the mutation results in a phenotype.

**Results:** Baseline calcium imaging showed significantly more patient hiPSC-CMs displayed calcium transient abnormalities compared to control hiPSC-CMs (66.7% vs 28%). 52.6% of patient hiPSC-CMs which displayed normal transients at baseline developed transient abnormalities in response to isoproterenol whilst only 19% of control hiPSC-CMs developed abnormalities. More patient hiPSC-CMs displayed spontaneous calcium transients at lower external calcium concentrations suggesting a lower threshold for store overload-induced calcium release (SOICR). The application of carvedilol corrected baseline calcium transient abnormalities in 80% of patient hiPSC-CMs. Allele-specific qPCR showed expression of both RYR2 alleles, suggesting that the p.(Arg4790\*) mutation does not result in haploinsufficiency.

**Conclusions:** The patient hiPSC-CMs display an abnormal calcium handling phenotype with increased abnormalities at baseline and after application of isoproterenol, and a reduced threshold for SOICR. The abnormal calcium handling phenotype was rescued by carvedilol indicating a potential therapeutic approach to prevent arrhythmias.

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## P05.26

**The RYR2 p.R420W mutation causes premature sudden cardiac death**

*O. Abdel-Razek<sup>1</sup>, A. Collier<sup>2</sup>, S. Predham<sup>2</sup>, F. Curtis<sup>2</sup>, A. Bullen<sup>2</sup>, J. Houston<sup>2</sup>, T. Benteau<sup>2</sup>, S. Stuckless<sup>3</sup>, T. Young<sup>2</sup>, S. Connors<sup>4</sup>, K. Hodgkinson<sup>2,3</sup>;*

<sup>1</sup>*Memorial University, Department of Internal Medicine, St. John's, NL, Canada,*

<sup>2</sup>*Memorial University, Discipline of Genetics, St. John's, NL, Canada, <sup>3</sup>Memorial University, Unit of Clinical Epidemiology, St. John's, NL, Canada, <sup>4</sup>Memorial University, Department of Internal Medicine, Division of Cardiology, St. John's, NL, Canada.*

**Background:** Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an arrhythmia syndrome causing sudden cardiac death (SCD). A putative founder mutation in RYR2 (p.R420W) located in the cytosolic portion of the protein crucial for calcium channel regulation has been ascertained in Newfoundland. Juvenile deaths were ascribed to this mutation in a previous publication without DNA confirmation.

**Research Question:** Does the RYR2 p.R420W mutation affect survival in a well ascertained autosomal dominant, multiplex (5 generation) three pedigree population?

**Study population:** All persons born at an a-priori 50% risk from three pedigrees segregating the RYR2 p.R420W were ascertained (n=79). Individuals were included if the disease status of  $\geq 50\%$  of their sibship was known (n=66). We excluded 6 juvenile (< 9yrs) deaths (n=60). Affected individuals included mutation positive and/or obligate carriers (OC) and/or SCD under 50 years. Unaffected status was defined as mutation negative. The remainder were unknown (UK).

**Methods:** Genetic and clinical data from all available medical records were collected from family members. Of 60 individuals, 27 (45%) were affected (16 mutation positive, 6 OC and 5 SCD) and 26 (43%) were unaffected. The unaffected and UK groups were combined. Time to death was compared using Kaplan-Meier time to event analysis and multivariate Cox regression.

**Results:** Affected status was significantly associated with mortality: RR = 4 (95% CI: 1.1-14.5) and affected males died earlier than females RR=7 (95% CI 1.5-34.5).

**Conclusions:** The RYR2 p.R420W mutation significantly affects mortality, particularly for males. Six juvenile deaths (with unknown mutation status) have occurred in these families.

## P05.27

**Genetic testing in a consecutive series of young athletes with suspected catecholaminergic polymorphic ventricular tachycardia**

*P. Berne<sup>1</sup>, N. Marziliano<sup>1</sup>, D. Fiscella<sup>2</sup>, A. Fiscella<sup>3</sup>, M. Monne<sup>4</sup>, G. Piras<sup>4</sup>, R. Asproni<sup>5</sup>, G. La Delfa<sup>5</sup>, D. Occipinti<sup>5</sup>, P. A. Merlini<sup>6</sup>, G. Casu<sup>7</sup>;*

<sup>1</sup>*Presidio Ospedaliero "San Francesco", ASL3 Nuoro, UOC di Cardiologia, Nuoro, Italy,*

<sup>2</sup>*Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione "Garibaldi, S. Luigi-S. Currò, Ascoli-Tomaselli". Presidio Ospedaliero Garibaldi-Nesima-Catania. UO di Cardiologia, Catania, Italy, <sup>3</sup>Fondazione Floresta Longo, Catania, Italy, <sup>4</sup>Presidio Ospedaliero "San Francesco", ASL3 Nuoro, Laboratorio Specialistico di Oncoematologia, Nuoro, Italy, <sup>5</sup>Centro di Medicina dello Sport-FMSI, Catania, Italy, <sup>6</sup>Azienda Ospedaliera Ospedale Niguarda Cà Granda, IV Divisione di Cardiologia, Milano, Italy.*

**Background.** Cathecolaminergic polymorphic ventricular tachycardia (CPVT; MIM 619138) is a rare inheritable disorder due to mutations in RYR2, CASQ, CALM1 and TRDN genes. It can be both autosomal dominant (RYR2 and CALM1) or recessive (CASQ2 and TRDN). It is clinically characterised by adrenergically induced polymorphic ventricular tachycardia (VT) in absence of structural heart diseases. Presentation includes syncope or sudden cardiac death (SCD) in young patients. Exercise testing is the gold standard for the clinical diagnosis and genetic testing may unravel additional asymptomatic carriers within the family.

**Methods.** We present a consecutive series of 25 young athletes (aged less than 18) with clinical suspicion of CPVT after undergoing pre-participation testing for competitive sports. Clinical assessment included: physical examination, surface ECG, echocardiogram and exercise testing (Bruce protocol). After genetic counselling, molecular testing was started by means of Next Generation Sequencing (NGS) on an Ion PGM platform with a targeted-resequencing panel of 148 cardiac genes.

**Results and Conclusion.** After testing, we found 8 (32%) mutation-carriers of the RYR2 gene, 4 (16%) mutations-carriers of the CASQ2 gene and 2 (8%) mutation-carriers of the CALM1 gene. Cascade testing revealed additionally RYR2 (n=13), CASQ2 (n=7) and CALM1 (n=3) mutations carriers with (47%) and without (53%) clinical signs. The study proved that proper clinical for sport activity in the young athletes is of outmost importance in identifying potentially individuals at risk of life-threatening arrhythmias. However psychosocial evaluations must included in order to overcome the life-style modifications subsequent to the genetic testing for the probands and their relatives.

## P05.28

**Next-generation sequencing to identify genetic causes of dilated cardiomyopathies**

*J. Bernasovska<sup>1</sup>, I. Boronova<sup>1</sup>, I. Bernasovsky<sup>1</sup>, J. Kmec<sup>2</sup>;*

<sup>1</sup>*University of Presov, Presov, Slovakia, <sup>2</sup>Cardiocentre, Hospital of J.A. Reiman, Presov, Slovakia.*

Dilated cardiomyopathy (DCM) is most common form of heart muscle disease and a leading cause of congestive heart failure. The genetics of cardiomyopathies represents one of the most active areas for the research in molecular genetics. The aim of our study was genetic testing of 29 patients with diagnosed DCM using next generation sequencing technology. All probands underwent clinical examination and genetic analysis. The control group included thirty healthy asymptomatic subjects without cardiovascular disease or a family history of DCM. Genomic DNA was extracted from whole peripheral blood using the standard extraction kit. Exome libraries were sequenced on a SOLiD 5500xl flowchip in paired end mode (75plus 25bp). Molecular-genetic analyses included also 50 different genes implicated in causing DCM. We didn't find any causal mutation in these genes in patients with dilated cardiomyopathy. On the other hand, we found missense mutations in other genes: SCN5A, SLC9A5, SLC26A9, SLC8A3 and MEGF8. In six patients with dilated cardiomyopathy was detected a disease causing mutation rs1805124, T/C in SCN5A gene. This mutation is disease specific for hypertrophic cardiomyopathy, however, the findings in the dilated cardiomyopathy has not yet been described. We recognised a novel missense mutation in SLC9A5 (NHE-5) gene, termed c.C826T:p.R276C (NM\_004594.2) and mapped to the chromosomal, region16:67,289,748 in three patients. Early diagnosis and correct assessment of the patient's risk profile plays a key role in determining the start of treatment. This study is the result of implementation of the projects APVV-0644-12 and ITMS 26220120241.

## P05.29

**Recurrent early-onset dilated cardiomyopathy and left ventricular noncompaction in a family with germline mosaicism for a MYH7 mutation**

L. Fernández<sup>1</sup>, P. Muñoz-Cabello<sup>1</sup>, K. Ibáñez<sup>1</sup>, V. Fdez-Montaño<sup>1</sup>, F. Nieto<sup>1</sup>, E. Mansilla<sup>1</sup>, F. Santos-Simarro<sup>1</sup>, J. Silla-Castro<sup>1</sup>, C. Labranderio<sup>2</sup>, M. Ortega<sup>2</sup>, J. Nevado<sup>1</sup>, S. García-Miñaur<sup>1</sup>, Á. del Pozo<sup>1</sup>, P. Lapunzina<sup>1</sup>, E. Vallespin<sup>1</sup>;

<sup>1</sup>INGEMM-IDIPAZ-HULP, Madrid, Spain, <sup>2</sup>Cardiology Department-IDIPAZ-HULP, Madrid, Spain.

**Introduction:** Dilated cardiomyopathy and left ventricular noncompaction are overlapping, genetically heterogeneous disorders with an average low diagnostic yield. Multigene testing may raise detection rate, while a larger increase in variants of uncertain significance hinders genetic counseling. We report a case of recurrent neonatal dilated cardiomyopathy with left ventricular noncompaction in two consecutive pregnancies from a healthy consanguineous couple that suggested autosomal recessive inheritance.

**Materials and Methods:** The index patient was studied with a custom-designed CardioMass v2.1 NGS panel including 268 genes associated with cardiomyopathies and cardiac rhythm disorders. Capture was based on Roche NimbleGen technology and sequencing was performed on a MiSeq Illumina platform. Family studies confirmed NGS variants by direct sequencing.

**Results:** An apparently de novo mutation in MYH7 in the two siblings disclosed germinal mosaicism in the family. Furthermore, both children, as well as their healthy parents, were carriers of a rare heterozygous variant in HCN4 which was previously described in association with bradycardia-tachycardia syndrome and left ventricular noncompaction.

**Conclusions:** These results highlight the usefulness of a NGS approach for genetically heterogeneous disorders. This case further shows the challenge of interpretation and the importance of familial segregation studies in the diagnosis of patients with multiple potentially pathogenic variants.

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## P05.30

**Identification of shear stress responsive enhancer elements in endothelial cells**

O. Bondareva<sup>1,2</sup>, R. Tsaryl<sup>2</sup>, A. Siekmann<sup>2</sup>, H. Schnittler<sup>1</sup>;

<sup>1</sup>Institute of Anatomy and Vascular Biology, Münster, Germany, <sup>2</sup>Max Planck Institute for Molecular Biomedicine, Münster, Germany.

Shear stress is a frictional force that significantly modulates endothelium structure and function due to change in metabolism, gene expression and posttranslational modifications. Unidirectional arterial shear stress levels are atheroprotective, supported by negative regulation of apoptotic signalling in vivo and in vitro. By contrast disturbed oscillatory flow acts as a pathological stimulus contributing to atherosclerosis and vasculopathies. Many studies demonstrated shear stress induced changes in gene expression, however the underlying transcriptional regulatory mechanisms remain unknown. In this study we aimed to identify shear stress responsive enhancer elements. Human umbilical vein endothelial cells were exposed to different shear stress profiles using the cone-and-plate based BioTechFlow System®. After application of 18 dyn/cm<sup>2</sup> unidirectional or ±3 dyn/cm<sup>2</sup> of oscillatory shear stress (0.5 and 6 hours stimulation) we performed chromatin immunoprecipitation of H3K27ac mark for active enhancers. Bioinformatical analysis of the sequenced immunoprecipitated DNA fragments revealed thousands of unique specific shear stress induced peaks corresponding to activated enhancers which were frequently found in proximity of genes regulated by shear stress. Further investigation of the peaks identified binding sites of several transcription factors implicated in shear stress responses and adaptation processes, e.g. SMADs for unidirectional and HIC1 for oscillatory flow. In conclusion, we demonstrate a genome-wide analysis of shear stress responsive regulatory elements that control the expression of genes important for shear stress-induced physiological and pathological endothelial phenotypes. The data provide a deeper insight into the diseases such as atherosclerosis and might open novel targets for therapy. Funded by CiM Flexible Funds, Münster, Germany.

## P05.31

**Familial Hypercholesterolaemia cascade genetic screening: A blind trial to evaluate the Randox FH Multiplex Arrays**

D. A. Bailie<sup>1</sup>, P. J. Hart<sup>1</sup>, M. J. Latten<sup>2</sup>, R. J. L. Martin<sup>1</sup>, T. L. Watson<sup>2</sup>, H. A. Murray<sup>2</sup>, J. V. Lamont<sup>2</sup>, S. P. Fitzgerald<sup>2</sup>, M. A. Crockard<sup>2</sup>, C. A. Graham<sup>1,2</sup>;

<sup>1</sup>Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust, Belfast, United Kingdom, <sup>2</sup>Randox Laboratories Ltd, 55 Diamond Road, Belfast, United Kingdom.

**Introduction:** Familial Hypercholesterolaemia (FH) is a common genetic disorder characterised by elevated low density lipoprotein cholesterol and early symptoms of coronary heart disease. Estimates suggest <10% of FH patients are diagnosed in most countries. Cascade genetic testing, as recommended by the National Institute for Health and Clinical Excellence (2008) and the European Atherosclerosis Society (2013), may successfully identify new cases. This study aims to determine the effectiveness of the Randox FH Multiplex Arrays for this purpose. This FH array is designed to detect 40 FH causing genetic alterations, accounting for 71% of mutations in Great Britain and Ireland.

**Materials and methods:** Close relatives (N=199) of mutation-positive index cases (previous confirmation by DNA sequencing) were selected for genetic testing. Screening was conducted as a blind study to remove bias. Mutational analysis was performed using the FH Multiplex Arrays I & II and the Evidence Investigator analyser (Randox Laboratories Ltd, Crumlin, UK).

**Results:** A true positive rate of 97.8% (89/91 patients) and a true negative rate of 100% (108/108 patients) demonstrates the sensitivity and specificity of the test in detecting 37 of 40 targeted family mutations present on the arrays, suggesting a concordance rate of 99% for the Randox FH Multiplex Arrays for cascade genetic screening.

**Conclusion:** The FH arrays successfully identify the most prevalent mutations in the Great Britain and Ireland cascade screening population. This will enable a cost effective method for targeted identification of people affected by FH, permit enhanced FH monitoring/management thus reducing FH associated morbidity and mortality.

## P05.32

**Rare variants in PCSK9 protect against familial hypercholesterolaemia in a patient with a pathogenic LDLR mutation**

S. Rodriguez-Novoa<sup>1</sup>, C. Rodriguez<sup>2</sup>, K. Heath<sup>1</sup>, A. del Pozo<sup>1</sup>, M. Tress<sup>2</sup>, E. Vallespin<sup>1</sup>, P.

Lapunzina<sup>1</sup>, P. Martínez<sup>3</sup>;

<sup>1</sup>INGEMM-Hospital La Paz, Madrid, Spain, <sup>2</sup>CNIO, Madrid, Spain, <sup>3</sup>Medicina Interna-Hospital La Paz, Madrid, Spain.

**Introduction:** Familial Hypercholesterolaemia (FH) is characterized by elevated low density lipoproteins (LDL-c) leading to premature cardiovascular disease. FH is caused by lost-of-function variants in LDLR, APOB and gain-of-function variants in PCSK9. Lost-of-function PCSK9 variants lead to decreases in LDL-c. Herein, we describe a family with a LDLR pathogenic mutation and two rare PCSK9 variants and their phenotype consequence.

**Patients and Methods:** LDLR, APOB, PCSK9 and LDLRAP1 were analysed in a FH family, by NGS and MLPA.

**Results:** A heterozygous known pathogenic LDLR mutation, c.2479G>A was observed in the proband and two offspring (Table). Interestingly, son 3 had normal-LDLc levels despite having the LDLR mutation. Two variants in PCSK9: [c.137G>T; c.2023delG] + [c.137G>T] was also detected. PCSK9:c.137G>T is a previously described lost-of-function variant associated with decreases in LDL-c. PCSK9:c.2023delG; p.Val675Leufs\*130 is a rare variant in the C-terminal, identified in a single individual in ExCA. In silico analyses predict the absence of two conserved Cysteines, critical for the correctly folding of the protein, therefore leading to a lost-of-function.

**Table.** Phenotype and genotype at LDLR and PCSK9 found in the family.

	LDL levels	LDLR genotype	PCSK9 Genotype	
Proband	High-LDLc	c.2479G>A; p.Val827Ile	c.137G>T; p.Arg46Leu	c.2023delG;
Daughter 1	High-LDLc	Yes, htz	Yes, htz	p.Val675Leufs*130
Daughter 2	Normal-LDLc	No	Yes, htz	
Son 3	Normal-LDLc	Yes, htz	Yes, hmz	Yes, htz

**Discussion:** The presence of lost-of-function variants in PCSK9 could ameliorate the effect of LDLR variants associated with FH. The counteracting effects of different genes should be explored in FH patients in order to improve genetic counselling.

## P05.33

### Next generation sequencing of familial hypercholesterolaemia-related genes in a Mediterranean European cohort

P.F. Dingli<sup>1</sup>, R. Attard, K. Cassar, R. Farrugia, S. Bezzina Wettinger<sup>2</sup>,  
Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, Msida, Malta.

**Introduction:** The aim of this study was to identify the polymorphisms responsible for familial hypercholesterolaemia in the Maltese population.

**Methods:** Dutch Lipid Clinic Network scores were calculated for each research subject in the Maltese Acute Myocardial Infarction Study which included 423 first myocardial infarction cases, 210 relatives of cases and 465 controls. Three cases had probable Familial Hypercholesterolemia (FH) and 67 cases had possible FH. All probable FH subjects and three samples from the list of possible FH were selected, the latter, based on LDL level. Libraries for FH were prepared using the SureSelect target enrichment protocol (Agilent Technologies) and sequenced on an Illumina HiSeq 4000. Next-GENEe® software was used to read data and obtain a list of variants.

**Results:** A summary of the SNPs found in LDLR, LDLRAP, APOB and PCSK9 is provided in table 1.

Mutation Function	APOB	LDLR	LDLRAP1	PCSK9
In-Frame	1			2
Missense	31	1	5	13
Noncoding	60	84	88	136
Synonymous	6	29	5	12
<b>Total</b>	<b>98</b>	<b>114</b>	<b>98</b>	<b>163</b>

Table 1. SNPs in chosen samples

Non-synonymous single nucleotide polymorphisms and polymorphisms in the adjacent introns were researched in known databases and in the literature. 4 candidate SNPs were short-listed – two in ApoB (P2739L, T98I) and two in PSCK9 L22\_L23insL, V474I)

## Conclusion

No strong mutation in known FH genes was observed indicating a polygenic cause for elevated LDL in these subjects.

## Funding

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## P05.34

### Parental somatic mosaicism in FBN1 and TGFBR1 genes in aortic pathology syndromes

P. Fernández Alvarez<sup>1</sup>, M. Borregan Prats<sup>1</sup>, L. Martínez Ribot<sup>1</sup>, M. del Campo Casanelles<sup>1</sup>, F. López Grondona<sup>1</sup>, M. Masas Castro<sup>1</sup>, A. Outeríral Adán<sup>1</sup>, G. Teixidó i Tura<sup>2</sup>, A. Evangelista Masip<sup>2</sup>, A. Cueto González<sup>1</sup>, T. Vendrell Bayona<sup>1</sup>, E. García-Arumí<sup>1,3</sup>, E. Tizzano Ferrari<sup>1,3</sup>,

<sup>1</sup>Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>2</sup>Department of Cardiology, Marfan Unit, Hospital Vall d'Hebron, Barcelona, Spain, <sup>3</sup>Biomedical Network Research Centre on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.

Molecular studies in patients with aortic pathology syndromes such as Marfan and Loeys-Dietz syndromes include NGS of FBN1, TGFBR1 and TGFBR2 as well as their parents to determine the origin of the mutation. Recent reports have shown that somatic mosaicism in parents of individuals with autosomic monogenic genetic diseases is more common than previously thought. Thus, an important proportion of de novo mutations could be the consequence of low-level mosaicism in one of the parents.

We analyzed 44 parents of patients considered as de novo mutations in FBN1 (n=42) and TGFBR1 (n=2) in order to detect somatic mosaicism. PCR amplicons for the exons of interest in each case were obtained followed by NexteraXT technology to generate libraries and deep sequencing using MiSeq sequencer. The obtained data were analyzed using a somatic alignment to detect low-frequency mosaisms.

In 3 index cases we found low-frequency mosaicism in one of their parents (in DNA blood sample and buccal cell swab and urine when available).

Gene	Mutation	origin	%blood DNA	% buccal swab DNA	% urine DNA
FBN1	c.1664G>A (p.Cys555Tyr)	paternal	19.13%	17.69%	In process
FBN1	c.425G>A (p.Gly142Glu)	maternal	13.02%	In process	In process
TGFBR1	c.683_685del (p.Glu228del)	paternal	4.52%	8.48%	4.36%

According to our results almost 10% of the de novo FBN1 mutations could

be the result of parental mosaicism. Thus, it is likely a more common occurrence than the expected in genes related with aortic pathology. Detection of these cases improves genetic counseling and allow the parents a cardiac follow up given that the somatic mutation may be expressed in the aortic tissue.

## P05.35

### PHACTR1 is a genetic susceptibility locus for fibromuscular dysplasia

S. R. Kiando<sup>1,2</sup>, N. R. Tucker<sup>3,4</sup>, L. Castro-Vega<sup>1,2</sup>, A. Katz<sup>5</sup>, V. D'Escamard<sup>6</sup>, C. Treard<sup>1,2</sup>, D. Fraher<sup>3,4</sup>, J. Albuison<sup>1,2,7</sup>, D. Kadian-Dodov<sup>6</sup>, Z. Ye<sup>8</sup>, E. Austin<sup>9</sup>, M. Yang<sup>5</sup>, K. Hunker<sup>5</sup>, C. Barlassina<sup>9</sup>, D. Cusi<sup>10</sup>, P. Galan<sup>11</sup>, J. Empana<sup>1,2</sup>, X. Jouven<sup>1,2,12</sup>, A. Gimenez-Roqueplo<sup>1,2,7</sup>, P. Bruneval<sup>1,2,13</sup>, E. S. H. Kim<sup>14</sup>, J. W. Olin<sup>6</sup>, H. L. Gornik<sup>14</sup>, M. Azizi<sup>15,16</sup>, P. Plouin<sup>1,2,15</sup>, P. T. Ellinor<sup>3,4</sup>, I. J. Kullo<sup>9</sup>, D. J. Milan<sup>3,4</sup>, S. K. Ganesh<sup>5</sup>, P. Boutouyrie<sup>1,2,17</sup>, J. C. Kovacic<sup>6</sup>, X. Jeunemaitre<sup>1,2,7</sup>, N. Bouatia-Naji<sup>1,2</sup>,

<sup>1</sup>INSERM, UMR970 Paris Cardiovascular Research Center (PARCC), Paris, France, <sup>2</sup>Paris-Descartes University, Sorbonne Paris Cité, Paris, France, <sup>3</sup>Cardiovascular research Center, Massachusetts General Hospital, Charlestown, MA, United States, <sup>4</sup>Program in Medical and Population Genetics, The Broad Institute of Harvard and MIT, Cambridge, MA, United States, <sup>5</sup>Department of Internal Medicine and Department of Human

Genetics, University of Michigan, Ann Arbor, MI, United States, <sup>6</sup>The Zena and Michael A. Wiener Cardiovascular Institute, Icahn School of Medicine, Marie-Josée and Henry R. Kravis Cardiovascular Health Center at Mount Sinai, New York, NY, United States,

<sup>7</sup>AP-HP, Department of Genetics, Hôpital Européen Georges Pompidou, Paris, France,

<sup>8</sup>Department of Medicine, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN, United States, <sup>9</sup>Dept. of Health Sciences, Genomic and Bioinformatics Unit, Chair and

Graduate School of Nephrology, University of Milano, Division of Nephrology, San Paolo Hospital, Milano, Italy, <sup>10</sup>Institute of Biomedical Technologies, Italian National Centre of Research, Milano, Italy, <sup>11</sup>INSERM, Nutritional Epidemiology Research Group, Sorbonne-Paris-Cité, UMR University of Paris 13/Inserm U-557/INRA U-1125/CNAM UMR970

Paris Cardiovascular Research Center (PARCC), Bobigny, France, <sup>12</sup>AP-HP, Department

of Cardiology, Hôpital Européen Georges Pompidou, Paris, France, <sup>13</sup>AP-HP, Department

of Pathology, Hôpital Européen Georges Pompidou, Paris, France, <sup>14</sup>Department of

Cardiovascular Medicine, Cleveland Clinic Heart and Vascular Institute, Cleveland, OH, United States, <sup>15</sup>AP-HP, Department of Hypertension, Hôpital Européen Georges Pomp

Introduction: Fibromuscular dysplasia (FMD) is a nonatherosclerotic vascular

disease leading to stenosis, dissection and aneurysm affecting mainly the renal and cerebrovascular arteries. FMD is often an underdiagnosed cause

of hypertension and stroke, has higher prevalence in females (~80%) but its

pathophysiology is unclear.

Methods and Results: We analyzed ~26K common variants (MAF>0.05) generated by exome-chip arrays in 249 FMD patients and 689 controls.

We replicated 13 modestly associated loci ( $P<10^{-4}$ ) in 402 cases and 2,537

controls and confirmed an association between FMD and a variant in the

phosphatase and actin regulator 1 gene (*PHACTR1*). Three additional case-

control cohorts including 512 cases and 669 controls replicated this result

(overall OR=1.39;  $P=7.4\times 10^{-10}$ ; 1,154 cases and 3,895 controls). The top variant, rs9349379, is intronic to *PHACTR1*, a risk locus for coronary artery

disease, migraine, and cervical artery dissection. The analyses of geometrical

parameters of carotids from 2,500 healthy volunteers indicate higher

intima-media thickness ( $P=1.97\times 10^{-4}$ ) and wall to lumen ratio ( $P=0.002$ ) in

rs9349379-A carriers, suggesting indices of carotid hypertrophy previously

described in carotids of FMD patients. Immunohistochemistry detected

*PHACTR1* in endothelium and smooth muscle cells of FMD and normal

human carotids. The expression of *PHACTR1* by genotypes in primary hu-

man fibroblasts showed higher expression in rs9349379-A carriers (N=86,  $P=0.003$ ). *Phactr1* knockdown in zebrafish resulted in dilated vessels indi-

cating impaired vascular development.

Conclusions. We report the first susceptibility locus for FMD and provide

evidence for a complex genetic pattern of inheritance and indices of shared

pathophysiology between FMD and other cardiovascular and neurovascular

diseases.

## P05.36

### Abnormal folate metabolism is associated with metabolic syndrome components in spontaneous hypertensive rats (SHR)

V. Kozich<sup>1</sup>, M. Pravenec<sup>2</sup>, J. Krijt<sup>1</sup>, J. Sokolová<sup>1</sup>, V. Zídek<sup>2</sup>, V. Landa<sup>2</sup>, P. Mlejnek<sup>2</sup>, J. Šilhavý<sup>2</sup>, M. Šimánková<sup>2</sup>, V. Škop<sup>3</sup>, J. Trnovská<sup>3</sup>, L. Kazdová<sup>3</sup>, T. W. Kurtz<sup>4</sup>,

<sup>1</sup>Institute of Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, Prague, Czech Republic, <sup>2</sup>Department of Model Diseases, Institute of Physiology, Czech

Academy of Sciences, Prague, Czech Republic, <sup>3</sup>Center of Experimental Medicine, Department of Metabolism and Diabetes, Institute for Clinical and Experimental

Medicine, Prague, Czech Republic, <sup>4</sup>Department of Laboratory Medicine, University of California, San Francisco, CA, United States.

Background: Metabolic syndrome (MS) is a multifactorial common risk factor

for cardiovascular disease. A number of studies indicated that abnormal

metabolism of sulfur amino acids and folates is associated with MS compo-

nents.

Methods and results: We analyzed the role folate deficiency in pathogene-

sis of the metabolic syndrome in the spontaneously hypertensive rat (SHR) model. The SHRs fed a folate-deficient diet showed significantly reduced serum folate ( $104 \pm 5$  vs.  $11 \pm 1$  nmol/L) and urinary folate excretion ( $4.3 \pm 0.6$  vs.  $1.2 \pm 0.1$  nmol/16 h), ectopic fat accumulation, impaired glucose tolerance and increased systolic blood pressure. Next, by using BXH/HXB recombinant inbred strains derived from the spontaneously hypertensive rat (SHR) and Brown Norway progenitors we detected a deletion variant in the folate receptor 1 (Folr1) promoter resulting in decreased expression of Folr1 mRNA in the kidney. Analysis of the SHR.BN-chr.1 congenic strain confirmed that the Folr1 promotor variant in SHR cosegregates with reduced renal expression of the mRNA and renal folate reabsorption, decreased serum folate levels, ectopic fat accumulation, reduced muscle insulin sensitivity, and increased blood pressure. Transgenic rescue experiments performed by expressing a Folr1 transgene in the SHR ameliorated most of the metabolic disturbances.

Conclusions: These data support hypothesis that genetic and nutritional folate deficiency is associated with development of the metabolic syndrome in SHR model and constitutes a previously neglected mechanism that may contribute to increased risk for cardiovascular disease.

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#### P05.37

##### Molecular screening of GATA4 in a Moroccan population suffering from non-syndromic tetralogy of Fallot (TOF)

*I. El Bouchikhi<sup>1,2</sup>, K. Belhassan<sup>1</sup>, F. Moufid<sup>1,2</sup>, M. Iraqui Houssaini<sup>2</sup>, L. Bouguenouch<sup>1</sup>, I. Samri<sup>1</sup>, K. Ouldim<sup>1</sup>, S. Atmani<sup>2</sup>;*

<sup>1</sup>Medical Genetics and Oncogenetics Laboratory, Hassan II University Hospital, Fez, Morocco, <sup>2</sup>Laboratory of Microbial Biotechnology, Faculty of Sciences and Techniques, University of Sidi Mohammed Ben Abdellah, Fez, Morocco, <sup>3</sup>Medico-Chirurgical Unit of Cardio-pediatrics, Department of Pediatrics, Hassan II University Hospital, Fez, Morocco.

Introduction: Tetralogy of Fallot is one of the most common congenital heart disease (CHD) with an incidence of 7% of CHDs. This disorder consists of four anomalies: ventricular septal defect; over-riding aorta; right ventricular outflow obstruction and right ventricular hypertrophy. Several transcription factors were identified to be involved in non-syndromic Tetralogy of Fallot, notably *NKX2-5* and *GATA4*. The latter is a zinc-finger protein that regulates the down-stream target genes, through binding to the specific motif (T/A)GATA(A/G), in order to control cell proliferation in the developing heart. The aim of this study was to screen the *GATA4* mutations in series of Moroccan patients with Tetralogy of Fallot.

Materials and Methods: thirty-one patients were recruited in cardio-pediatric department of HASSAN II university hospital-Fez. After obtaining consents, we have collected blood samples, and extracted genomic DNA specimens. Then, we carried out PCR and direct sequencing of the *GATA4* coding regions for each patient. The obtained sequences were analysed by Bioinformatic alignment tool.

Results: we detected two missense mutations and five synonymous mutations. These mutations were clustered on exons two and five. In addition to eight polymorphisms spread over the intronic regions, in particular, introns one and four. Considering the non-synonymous mutations alone, *GATA4* mutation rate in our cohort reaches 6.5%.

Conclusions: given the important role of *GATA4* in regulating the heart development process, the identified mutation seems most likely to be responsible of the observed phenotype in the mutation carriers. Finally, to confirm these results a larger Moroccan TOF cohort will be pertinent.

#### P05.38

##### Relationship between AGT haplotype in ITGA4 gene and antibody-mediated rejection after heart transplantation.

*G. Marron-Linares<sup>1</sup>, L. Nunez<sup>1</sup>, M. Crespo-Leiro<sup>2</sup>, N. Suarez-Fuentetaja<sup>1</sup>, E. Barge-Caballero<sup>2</sup>, M. Paniagua-Martin<sup>2</sup>, Z. Grille-Cancela<sup>2</sup>, J. Muñiz-Garcia<sup>1</sup>, E. Rodríguez<sup>3</sup>, J. Vázquez<sup>2</sup>, M. Hermida-Prieto<sup>1</sup>;*

<sup>1</sup>Grupo de investigación en Cardiología. Instituto de Investigación Biomédica de A Coruña (INIBIC). Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas. Universidad da Coruña (UDC), A Coruña, Spain, <sup>2</sup>Servicio de Cardiología. Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, A Coruña, Spain, <sup>3</sup>Department of Anatomic Pathology, Heart and Vascular Institute, Cleveland Clinic, Cleveland, OH, United States.

Introduction: Heart transplant (HT) is a life-saving treatment for patients with end-stage heart failure. A low incidence but significant problem in HT is the antibody-mediated rejection (AMR). In AMR, there is complement activation which contributes to graft damage.

Purpose: The aim of this study is to analyze if genetic variants in genes related to complements pathway could be associated with the development of AMR.

Methods: Genetic variants in 42 genes related to complement pathway have been analyzed by next generation sequencing (Trusight one sequencing panel-Illumina) in 48 HT patients, 24 with and 24 without AMR. Statistical was performed with SNPstats.

Results: 287 Single nucleotide polymorphisms (SNPs) in 42 genes were analyzed in both groups (AMR vs control). Three SNPs (rs1143674, rs7562325 and rs1143676) in ITGA4, which codifies for integrin alpha-4 protein, showed significant association with AMR. Haplotype-association analysis, comprising these 3 SNPs, showed that there is a significant association between AGT haplotype and AMR (OR (95%)=9.33 (2.14-40.58) p=0.0047). Other three SNPs (rs1899450\_MBL2, rs1048118\_CFP, and rs3774268\_MASP1) also showed significant association with AMR (p<0.05).

Conclusions: AGT haplotype in ITGA4 gene and rs1899450\_MBL2, rs1048118\_CFP, and rs3774268\_MASP1 could be associated with development of AMR in HT patients. However, more research must be done to determine the possibly role of these variants in the development of AMR.

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#### P05.39

##### Rare synonymous variants in LDLR gene are associated with Familial Hypercholesterolaemia

*C. Rodríguez Antolín<sup>1</sup>, C. Rodríguez Jiménez<sup>1</sup>, L. Reinares<sup>2</sup>, F. Arrieta<sup>3</sup>, E. Vallespín<sup>1</sup>, P. Lapunzina<sup>1</sup>, S. Rodríguez Noya<sup>1</sup>;*

<sup>1</sup>INGEMM, Madrid, Spain, <sup>2</sup>Hospital Universitario Clínico San Carlos, Madrid, Spain,

<sup>3</sup>Hospital Universitario Ramón y Cajal, Madrid, Spain.

INTRODUCTION: Familial hypercholesterolaemia (FH) is an autosomal dominant disease caused by mutations in LDLR, APOB, PCSK9, characterized by high levels of LDL-c increasing the risk of cardiovascular disease. About 35-40% of FH-individuals lack of mutations. Herein we analyse the synonymous variants found in individuals without genetic confirmation of the disease.

PATIENTS AND METHODS: Individuals with possible/confirmed clinical diagnosis of FH were remitted to our service for genetic testing. LDLR, APOB, PCSK9 and LDLRPA1 was analysed by Next Generation Sequence (NGS). MLPA was performed to detect large insertion/deletions in LDLR. Synonymous variants with frequency <0.01 were selected for in silico analyses.

RESULTS: Rare synonymous variants were found in 12 out of 100 patients. The synonymous LDLR:c.2388C>T;p.Ile759Ile, with allele frequency of 0.00001 in ExCA, is located at the -2 position of the splice donor site within exon-16. In silico analyses using Human Splicing Finder identified a new site in enhancer motifs (ESE) and other splicing motifs predicting an impact in protein expression. Ex-SKIP revealed the mutated allele has a higher chance of exon skipping than wild type. ESEfinder predicts the lost of two junction sites to SRF1 in the mutated allele with respect to wild type. These data suggest this variant might affect critical regulatory sequences which could change the primary structure protein.

CONCLUSIONS: Rare synonymous variants should be analysed in patients without genetic confirmation of FH especially those with any predicted impact on protein regulation. Further studies should be performed in order to dilucidated the mechanism proposed for pathogenicity of LDLR:c.2388C>T;p.Ile759Ile.

#### P05.40

##### A genome-wide association study for hypertriglyceridemia in ethnic Saudi Arabs

*M. M. Alrasheed<sup>1</sup>, S. M. Waki<sup>2</sup>, R. Ram<sup>3</sup>, N. P. Muiya<sup>2</sup>, G. Moraham<sup>4</sup>, B. F. Meyer<sup>2</sup>, N. Dzimir<sup>2</sup>;*

<sup>1</sup>College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, <sup>2</sup>King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia, <sup>3</sup>Western Australian Institute for Medical Research, University of Western Australia, Australia, Perth, Australia, <sup>4</sup>Western Australian Institute for Medical Research, University of Western Australia, Perth, Australia.

Introduction: Hypertriglyceridemia (hTG) is a primary cause for coronary artery disease (CAD), with a strong genetic component. However the genetic basis for hTG is not fully understood yet.

Materials and Method: In the present study, we performed a common variant association study (CVAS) using the Affymetrix High Density Axiom platform on the disease in the ethnic Saudi Arab population (927 cases versus 3159).

Results: The lead variant was the rs1558861 [1.99(1.73-2.30); p=7.37x10-22], residing on chromosome (chr) 11 at the apolipoprotein A-1 (APOA1) locus. The rs780094 [1.34(1.21-1.49); p=8.57x10-8] on chr 2 at the

glucokinase regulatory protein (GCKR) locus was similarly significantly associated, while the rs10911205 [1.29(1.16-1.44); p=3.52x10-6] on chr 1 at the laminin subunit gamma-1 (LAMC1) locus showed suggestive association with disease. Furthermore, the rs17145738 [0.68(0.60-0.77); p=6.69x10-9] on chr7 at the carbohydrate-responsive element-binding protein-encoding (MLXIPL) gene locus displayed significant protective characteristics, while another variant rs6982502 [0.76(0.68-0.84); p=5.31x10-7] on chr8 showed similar but weaker properties. These findings were replicated in 317 cases versus 1415 controls from the same ethnic Arab population.

Conclusion: Our data reveal several variants across the human genome that are associated with hTG in ethnic Arabs.

#### P05.41

#### Impact of renin-angiotensin-aldosterone polymorphisms and mitochondrial haplogroups in phenotypic expression of hypertrophic cardiomyopathy in genotyped patients

I. Perez<sup>1</sup>, M. Sabater<sup>1</sup>, E. García-Molina<sup>1</sup>, C. Muñoz<sup>2</sup>, M. Navarro<sup>2</sup>, M. Olmo<sup>2</sup>, D. López<sup>2</sup>, J. Gimeno<sup>2</sup>;

<sup>1</sup>Hospital Clínico Universitario Virgen de la Arrixaca. Inherited cardiomyopathies unit, Murcia, Spain, <sup>2</sup>University Hospital Virgen de la Arrixaca, Department of Cardiology, Murcia, Spain.

**Introduction:** Hypertrophic Cardiomyopathy (HCM) is a cardiovascular condition, with a variable penetrance and expression. The renin-angiotensin-aldosterone system (RAAS) and mitochondrial DNA (mtDNA) inherited variants have been associated with left ventricular hypertrophy and play a role in phenotype of HCM.

**Purpose:** To study the role of RAAS polymorphisms and mitochondrial haplogroups on the HCM clinical expression in a population of patients with HCM and genetic diagnosis.

**Methods:** Study population comprised 156 patients with HCM carrying a causative mutation in a sarcomeric MYBPC3 and MYH7 gene. All individuals underwent cardiac examination. We genotyped six RAAS polymorphisms and six mitochondrial single nucleotide polymorphisms (SNP's) which define the most common European haplogroups.

**Results:** On multivariable analysis, CMA polymorphism (-1903A) carriers showed a phenotype characterised by systolic impairment and left ventricular dilatation. The DD-ACE was associated with a lower survival free from AF and stroke. Polymorphism T174M, was related to a higher hypertrophy. The mitochondrial haplotype V was associated with a reduced survival free from AF and limiting dyspnoea. Haplotype U was associated to dyspnoea. Carriers of haplotype J had more often systolic impairment. Haplotype HV was associated with non sustained ventricular tachycardia and sudden death risk score.

**Conclusions:** Our data suggest that the genetic variation at some RAAS SNP's could contribute to the different expression in HCM. Moreover, mtDNA haplotypes may have the potential of becoming biomarkers in cardiomyopathy.

SNP's	Variable	p
(-1903A)	Systolic impairment	<0.001
(-1903A)	Left ventricular dilatation	0.02
DD-ECA	Survival free from AF	0.017
DD-ECA	Stroke	0.032
AGT (T174M)	IMLVWT	0.022
Mitochondrial Haplotype	Variable	p
V	Survival free from AF	0.029
V	NYHA III-IV	0.002
U	NYHA III-IV	0.05
J	Systolic impairment	0.012
HV	NSVT	0.031
HV	Risk Score	0.015

#### P05.42

#### Mutation analysis of Hypertrophic Cardiomyopathy genes using Next-Generation Sequencing

A. M. Coutinho, J. Tavares, M. Carmo-Fonseca;

GenoMed, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

**Introduction:** Hypertrophic Cardiomyopathy (HCM) is a common autosomal dominant genetic disease associated with sudden death and progressive heart failure. Genetic testing is routinely offered to these patients as a means to improve prognosis through appropriate lifestyle and medical interventions. However, because mutations in at least 30 genes have been linked to HCM, conventional diagnosis by Sanger sequencing is laborious and time consuming. Advances in high throughput sequencing technologies have the potential to solve this problem, but they also raise new challenges. The aim of this study was to develop a Next-Generation Sequencing (NGS) approach to screen for mutations in patients with this disorder.

**Methods:** The study population comprised 60 unrelated consecutively eva-

luated patients diagnosed with HCM. A capture library was designed and target regions were sequenced on an Illumina platform. The study was designed to screen the coding DNA sequence of either 20, 46 or 57 genes implicated in HCM and other inherited cardiomyopathies: ABCC9, ACTC1, ACTN2, BAG3, CASQ2, CAV3, CHRM2, CRYAB, CSR3, DES, DMD, DOLK, DSC2, DSCG2, DSP, DTNA, EMD, FHL2, GATAD1, GLA, ILK, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEXN, PDLIM3, PKP2, PLN, PRKAG2, PTPN11, RAF1, RBM20, RYR2, SCN5A, SGCD, TAZ, TCAP, TMEM43, TNNC1, TNNT3, TNNT2, TPM1, TRDN, TTN, TTR and VCL.

**Results:** Pathogenic mutations were detected in 17 patients (28.3%).

**Conclusions:** We developed an NGS procedure for screening mutations in 57 genes associated with HCM. The advantage of expanding the number of genes screened for mutations will be discussed.

#### P05.43

#### Clinical application of a whole genome sequencing (WGS) approach to Inherited Cardiac Disorders

P. A. James<sup>1</sup>, T. Thompson<sup>1</sup>, D. Du Sart<sup>2</sup>, R. Leach<sup>3</sup>, D. Floeke<sup>3</sup>, R. Tearle<sup>3</sup>, D. Zentner<sup>1</sup>, A. Trainer<sup>1</sup>, J. Vohra<sup>1</sup>, I. Winship<sup>1</sup>;

<sup>1</sup>Royal Melbourne Hospital, Melbourne, Australia, <sup>2</sup>Victorian Clinical Genetic Service, Melbourne, Australia, <sup>3</sup>Complete Genomics, Mountain View, CA, United States.

**Background:** Identifying the underlying mutation in families affected by inherited cardiac disorders allows optimal screening and risk management but in practice this remains unachievable in many families reflecting the complexity of these disorders. We examined the potential of whole genome sequencing (WGS) compared to current standard practice in the setting of a multidisciplinary cardiac-genetics clinic (CGC).

**Methods:** Living index cases assessed in the CGC (n=100) were consented for WGS alongside usual care. The genome was sequenced and annotated (Complete Genomics). Variants were filtered against public databases and data from healthy elderly controls. Clinical interpretation was applied to high quality calls for SNPs, InDels, structural variants and CNVs. To manage the scope of unintended findings analysis and validation was limited to genes associated with a cardiac phenotype (HPO database).

**Results:** Analysis of 75 genomes has been completed (36 Cardiomyopathy, 31 Familial Arrhythmias, 5 Aortopathy) revealing an average of 125 protein-altering variants per genome in genes with a reported cardiac phenotype. For 21 individuals (28%) a potentially causative mutation was detected in standard care. This increased to 42 individuals (56%) following WGS (p<0.001). In 12% of cases more than one likely pathogenic change was detected, while 46 (61%) carry clinically suspicious variants that are currently unclassifiable. The broad genetic data allowed the additional identification of undiagnosed syndromic disorders and novel candidate genes where multiple truncating mutations were observed in highly constrained genes.

**Conclusion:** While highly complex, whole genome sequencing achieved a significant improvement in genetic diagnosis over standard investigation and gene testing.

#### P05.44

#### Correlation with platelet parameters and genetic markers of thrombophilia panel (factor II g.20210G>A, factor V Leiden, MTHFR (C677T, A1298C), PAI-1, $\beta$ -fibrinogen, factor XIIIa (V34L), glycoprotein IIIa (L33P)) in ischemic strokes

S. Tasdemir<sup>1</sup>, H. B. Erdem<sup>1</sup>, I. Sahin<sup>1</sup>, L. Ozel<sup>2</sup>, G. Ozdemir<sup>2</sup>, R. Eroz<sup>3</sup>, A. Tatar<sup>1</sup>;

<sup>1</sup>Ataturk University Department of Medical Genetics, Erzurum, Turkey, <sup>2</sup>Ataturk University Department of Neurology, Erzurum, Turkey, <sup>3</sup>Duzce University Department of Medical Genetics, Duzce, Turkey.

**Introduction:** Ischemic stroke, an important type of arterial thrombosis, is associated with increased mortality risk, severe disability and life quality impairment. In this study we analyzed mean platelet volume (MPV), platelet count (PLT) values and genetic thrombophilia markers of patients who have ischemic stroke history and searched the relationship with genetic predisposition of ischemic stroke and platelet parameters.

**Materials and Methods:** A retrospective, clinical trial was performed by reviewing the ischemic stroke history (except cryptogenic events) of 599 patients, and 100 controls. The results of the genetic thrombophilia panel were used to classify the study group and control group into low and high risk for thrombophilia groups. The high-risk group included patients homozygous/heterozygous for factor II g.20210G>A or factor V Leiden mutations with/without any other polymorphism. The low-risk group included patients heterozygous or homozygous for MTHFR (C677T, A1298C), PAI-1,  $\beta$ -fibrinogen, factor XIIIa (V34L) and glycoprotein IIIa (L33P) polymorphisms or negative in terms of both mutations and polymorphisms.

Results: High risk group mutations were significant; but low risk group polymorphisms were not significant for ischemic stroke. There was significant difference between MPV and PLT values of ischemic stroke and control group.

Conclusion: Factor II g.20210G>A and factor V Leiden mutations are important risk factors for ischemic stroke. Thrombophilia mutations and polymorphisms do not have a significant effect on MPV and PLT values in ischemic stroke patients.

Summary of descriptive statistics for the groups.								
	n	Mean PLT $\pm$ SD	p	Mean MPV $\pm$ SD	p	Mutation score	p	
Patients	High risk group	139	267 $\pm$ 68	0.014a 0.392b 0.048c	8.4 $\pm$ 1	0.000a 0.365b 0.000c	0.275d	
	Low risk group	460	268 $\pm$ 65			8.4 $\pm$ 1		
	Control	100	251 $\pm$ 57			7.8 $\pm$ 0.8	3.2	

## P05.45

### New mutation c.IVS2+45T>G in LMNA gene causing aberrant splicing and leading to cardiac conduction defect and variable myopathy

*Y. Rogozhina<sup>1</sup>, S. Mironovich<sup>1</sup>, A. Shestak<sup>1</sup>, T. Adyan<sup>2</sup>, A. Bakulina<sup>3</sup>, A. Polyakov<sup>2</sup>, E. Zaklyazminskaya<sup>1,4</sup>,*

<sup>1</sup>Petrovsky Russian Research Center of Surgery, Moscow, Russian Federation, <sup>2</sup>Research Centre of Medical Genetics, Moscow, Russian Federation, <sup>3</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>4</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Introduction: Most of mutations in LMNA gene are unique and were found in 1-2 unrelated families. Interpretation of new genetic findings especially beyond the coding area and canonical splice sites can be difficult and requires advanced investigation.

Materials and Methods: The clinical and molecular-genetic study was performed for the patient with cardiac conduction defect and variable myopathy. Genetic study included: DNA-testing in LMNA gene by Sanger sequence. The prevalence of new genetic variant was tested in cohort of EDMD patients (99 DNA samples) by MLPA, and in control group (100 healthy volunteers) by RFLP method. Potential influence of c.IVS2+45T>G was performed by open bioinformatics tools. Bidirectional Sanger sequencing followed non-quantitative RT-PCR of target fragment of LMNA cDNA was performed. In silico modeling was performed with CCBulder server and Modeller software. Results: Diagnosis EDMD was established in proband based on clinical investigation. Genetic testing revealed new intronic variant c.IVS2+45T>G in LMNA gene in proband and affected daughter but not in two healthy sons. Activation of cryptic splice site was predicted by open bioinformatics sources. Insertion of 45 bp was confirmed in proband's cDNA. The possible functional effects of aberrant protein were predicted.

Conclusion: New variant c.IVS2+45T>G in LMNA gene is pathogenic and causative for EDMD in this family. Deep intronic variants in the LMNA gene can be responsible for some genotype-negative EDMD cases.

## P05.46

### The burden of complex arrhythmic genotypes in a consecutive series of Sardinian population of patients with primary arrhythmic disorders.

*N. Marziliano<sup>1</sup>, P. Berne<sup>1</sup>, G. Viola<sup>1</sup>, V. Carboni<sup>1</sup>, D. Fiscella<sup>2</sup>, A. Fiscella<sup>3</sup>, P. A. Merlini<sup>4</sup>, F. Notarangelo<sup>5</sup>, N. Gaibazzi<sup>5</sup>, D. Ardissono<sup>5</sup>, G. Piras<sup>1</sup>, M. Monne<sup>1</sup>, R. Asproni<sup>1</sup>, G. Casu<sup>1</sup>,*

<sup>1</sup>Presidio Ospedaliero "San Francesco", ASL3 Nuoro, Laboratorio Specialistico di Oncoematologia, Nuoro, Italy, <sup>2</sup>Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione "Garibaldi, S. Luigi-S. Curro, Ascoli-Tomaselli". Presidio Ospedaliero Garibaldi-Nesima-Catania. UO di Cardiologia, Catania, Italy, <sup>3</sup>Fondazione Floresta Longo, Catania, Italy, <sup>4</sup>Azienda Ospedaliera Ospedale Niguarda Ca Granda, IV Divisione di Cardiologia, Nuoro, Italy, <sup>5</sup>Azienda Ospedaliero Universitaria di Parma, Parma, Italy.

Brugada syndrome (BrS), Long QT syndrome (LQTS), Short QT syndrome (SQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT), are primary inherited cardiac arrhythmic disorders that can lead to sudden death. Prevalence might change according to the ethnicity and affecting mainly male patients in their third to fourth decade of life. They are inherited as autosomal dominant traits; but recent studies identified complex thought rare genotypes.

We aimed to investigate the prevalence of mutations in the disease-genes underlying such inherited disorders in 400 patients of Sardinian ancestry.

We analysed -by means of NGS - 148 arrhythmogenic genes in a consecutive series of 400 Sardinian patients enrolled at the San Francesco Hospital from years 2014 to 2015. In addition, to identify genes significantly enriched in the Sardinian series, we performed a mutation burden test by using as control dataset of continental Italians.

We confirmed the genetic heterogeneity of the BrS, LQTS and CPVT but in addition we identified: a) a burden of complex genotypes (compound/double heterozygous and triple mutation carriers) with a higher prevalence in Sardinians compared with continental Italians (7.8% vs 3.2%) and b) new potential CPVT/LQTS candidates (such as CACNA1B, CASQ2) thus confirming the idea of a possible genetic overlap between the different disorders. As expected, the use of NGS in the Sardinian population to assess the contribution of genetics to such malignant cardiac traits, can further elucidate causal mechanisms and in addition these results identify a burden of complex genotypes that, in panels derived from more cosmopolitan populations, are missing.

## P05.47

### Identification of genomic region associated with Lipoprotein Cholesterol: Genome-wide association studies (GWAS) in Tehran Cardio-metabolic Genetic Study (TCGS)

*M. S. Fallah<sup>1,2</sup>, M. Daneshpour<sup>1</sup>, B. Sedaghatkhayat<sup>1</sup>, K. Guity<sup>1</sup>, F. Azizi<sup>3</sup>,*

<sup>1</sup>Cellular and Molecular Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Kawsar Human Genetics Research Center, Tehran, Iran, Islamic Republic of, <sup>3</sup>Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Introduction: Genome-wide association studies (GWAS) have become an important strategy for genetic examination of human complex diseases. LDL cholesterol (LDL-C) plays a proven effect as risk factor in the cardiovascular disease and needs further investigation to provide a better understanding of the pathogenesis. Here we report results of a GWAS in an Iranian population participating in Tehran cardio-metabolic genetic study (TCGS).

Material and Methods: GWAS data was used; including 699,950 quality-checked SNPs (HumanOmniExpress-24-v1-0 bead at deCODE genetics) from 8174 participants with measures of blood LDL-C concentrations (with normal distribution and excluding outlier data ( $\pm 4SD$ )). Efficient Bayesian mixed-model analysis with the help of Bolt-LMM v2.1 was used for association analysis in total population, male and female separately.

Results: At the genome-wide level ( $p < 10^{-7}$ ), 27 SNPs that was associated with LDL-C concentration in total population mostly in both sexes. Associated signals were concentrated in 19p13.2, 1p13.3, 2p24.1 and 5q13.3 genomic region.

Conclusion: In this study we discovered four loci with protein coding genes (LDLR, CELSR2, APOB and HMGCR) which confirm the previous reports in other ethnicities. This study is the first GWAS in Iran and results could provide better understanding of the underlying biological mechanism of LDL-C regulation and consequent using in therapeutic targets and clinical care for cardiovascular disease.

## P05.48

### A genome-wide association study for low HDL-cholesterol levels in ethnic Saudi Arabs

*N. P. Muiya<sup>1</sup>, S. M. Wakil<sup>1</sup>, R. Ram<sup>2</sup>, E. Andres<sup>1</sup>, N. Mazhar<sup>1</sup>, S. Hagos<sup>1</sup>, G. Morahan<sup>2</sup>, B. F. Meyer<sup>1</sup>, N. Dzimiri<sup>1</sup>,*

<sup>1</sup>King Faisal Spec. Hospital and Research Centre, Riyadh, Saudi Arabia, <sup>2</sup>Western Australian Institute for Medical Research, University of Western Australia, Australia, Perth, Australia.

Introduction: The genetic basis for changes in high density lipoprotein-cholesterol (HDL) levels is not fully elucidated yet. We performed a GWAS for this disorder in ethnic Arabs.

Methods: We employed the Affymetrix high density Axiom Genome-Wide ASI Array (Asian population) that provides high genetic coverage containing a total of 598,000 SNPs to genotype a total of 5495 individuals in the discovery and validation experiments.

Results: Two variants, the rs1800775 [1.31(1.22-1.42);  $p=3.41E-12$ ] on the CETP gene and rs359027 [1.26(1.16-1.36);  $p=2.55E-08$ ] on the LMCD1 gene were significantly associated with harbouring of low HDL-cholesterol. Furthermore, the rs3104435 [1.26(1.15-1.38);  $p=1.19E-06$ ] at the MATN1 locus, rs9835344 [1.16(1.08-1.26);  $p=8.75E-06$ ] in the CNTN6, rs1559997 [1.3(1.14-1.47);  $p=9.48E-06$ ] in the SDS and rs1670273 [1.2(1.1-1.31);  $p=4.81E-06$ ] at the DMN/SYNN gene locus exhibited suggestive association with the disease. Seven other variants including the rs1147169, rs10248618, rs476155, rs7024300, rs10836699, rs11603691 and rs750134 exhibited borderline protective properties. The validation and joint meta-analysis

experiment resulted in the rs1800775, rs3104435 and rs359027 retaining their predisposing properties, as well as the rs10836699 and rs11603691 showing protective properties.

Conclusions: Our data demonstrate association of several variants across the genome with changes in low HDL-cholesterol levels in ethnic Arabs.

#### P05.49

#### Genetic Testing for Marfan And Loeys-Dietz Syndrome - Utility of the Ghent Nosology as a clinical selection tool

J. Dean;

University of Aberdeen, Aberdeen, United Kingdom.

Marfan and Loeys-Dietz syndromes are aortopathies with additional connective tissue features. Marfan is associated with mutation in FBN1 and Loeys-Dietz syndrome with mutation in TGFBR1 and TGFBR2. The clinical diagnosis of Marfan syndrome is made using the International (Ghent) Nosology, originally described in 1996, and updated in 2010. Aortic dilatation or ectopia lentis are key features - other findings are aggregated to create the systemic score. A score of 7 or more contributes to a diagnosis of Marfan. The nosology does not help directly to diagnose Loeys-Dietz syndrome. 77% of probands attending the Aberdeen Marfan Clinic who fulfil the Ghent 2010 nosology for a diagnosis of Marfan syndrome, and 74% who fulfil Ghent 1996 have an FBN1 mutation. In probands who do not fulfil the nosology, 16% of those with aortic dilatation and any systemic score below 7 have TGFBR1 or TGFBR2 mutations, but none in this group have FBN1 mutation. In those with no ocular features, and no aortic findings, around 20% of those with a systemic score of 5 have FBN1 mutations, but few cases with scores below this have mutations. This case series suggests it is worth considering Marfan syndrome and testing for FBN1 in probands with a systemic score of 5 or greater under Ghent 2010 but no cardiac or ocular findings, and that probands with aortic dilatation but a systemic score of less than 7 are more likely to have Loeys-Dietz than Marfan.

#### P05.51

#### Ventricular fibrillation as presenting symptom in an adolescent with Marfan syndrome

A. Wischmeijer<sup>1,2</sup>, V. Di Pierro<sup>1</sup>, E. Micaglio<sup>1</sup>, M. Ziacchi<sup>3</sup>, G. Gargiulo<sup>4</sup>, D. Pacini<sup>5</sup>, G. Tortora<sup>1,6</sup>, G. Severi<sup>1</sup>, E. Mariucci<sup>7</sup>, L. Lovato<sup>8</sup>, M. Seri<sup>1</sup>, C. Rossi<sup>1</sup>, M. Bonvicini<sup>7</sup>;

<sup>1</sup>Medical Genetics Unit, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy, <sup>2</sup>Genetic Counselling Service, Department of Pediatrics, Regional Hospital of Bolzano, Bolzano, Italy, <sup>3</sup>Institute of Cardiology, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy, <sup>4</sup>Pediatric Cardiac Surgery Unit, Policlinico S. Orsola-Malpighi, Università degli Studi, Bologna, Italy, <sup>5</sup>Cardiac Surgery Unit, Policlinico S. Orsola-Malpighi, Università degli Studi, Bologna, Italy, <sup>6</sup>Clinical Immunology Unit, AOU Riuniti Ancona, Università Politecnica delle Marche, Ancona, Italy, <sup>7</sup>Pediatric Cardiology and GUCH Unit, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy, <sup>8</sup>Cardio-Thoracic Radiology Unit, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy.

Marfan syndrome (MFS) is a highly variable, autosomal dominant condition, mainly characterized by ocular, cardiovascular and musculo-skeletal manifestations. Aortic root dilatation and mitral valve prolapse are the most common cardiovascular signs and source of major clinical concern because they can lead to severe complications. Arrhythmias such as atrial flutter and ventricular dysrhythmias have been described in MFS, but currently available knowledge is limited.

We report on a 13-year old female who presented with several unexplained episodes of ventricular dysrhythmia/fibrillation; recurrent myocarditis and cardiomyopathy were excluded and an ICD was positioned. Previously, she underwent corrective surgery for inguinal hernia and flat feet. Pre-operative ECGs resulted normal. Additional clinical investigations revealed abnormal ECGs, severe aortic root dilatation, mitral valve prolapse, mild pectus carinatum and scoliosis, arachnodactyly and mild craniofacial MFS signs, while ectopia lentis and myopia were excluded.

Molecular genetic analysis of FBN1 (HRM and MLPA), TGFBR1/2, SMAD3 and TGFB2 for suspected MFS or Loeys-Dietz syndrome resulted negative. Therefore, whole exome sequencing (WES) was performed which led to the identification of a de novo heterozygous nonsense mutation in FBN1 (NM\_000138 c.7800C>G p.Tyr2600X), previously described in a subject with Marfan syndrome (UMD-FBN1 mutations database ID 207), and absent in genetic databases of general population cohorts (1000 genomes, ExAC databases).

To our knowledge, this is the first description of ventricular fibrillation as presenting symptom in a pediatric MFS patient. This case confirms that potentially life-threatening arrhythmias should be considered in MFS. Large multi-centre studies are needed to gain information on this issue.

#### P05.52

#### MicroRNA-625-5p as a novel candidate modulator of AMPK pathway in statin-treated individuals without coronary artery disease

N. Coban<sup>1</sup>, D. Pirim<sup>2</sup>, A. Erkan<sup>3</sup>, F. Guclu-Geyik<sup>4</sup>, B. Ekici<sup>3</sup>, N. Erginol-Unaltuna<sup>4</sup>;

<sup>1</sup>Institute for Experimental Medical Research, Department of Genetics, Istanbul University, Istanbul, Turkey, <sup>2</sup>Department of Molecular Biology and Genetics, Marmara University, Istanbul, Turkey, <sup>3</sup>Department of Cardiology, Medical Faculty, Ufuk University, Ankara, Turkey, <sup>4</sup>Institute for Experimental Medical Research, Department of Genetics, Istanbul, Turkey.

Aim: AMP-activated protein kinase (AMPK) is a signal molecule that phosphorylates and inactivates 3-hydroxy-3-methylglutaryl coenzyme A reductase, the key enzyme controlling cholesterol biosynthesis. We aimed to test whether AMPK signaling pathway is effective in human macrophages which play crucial role in atherosclerosis. In addition, to identify novel modulators of AMPK pathway while exploring the effect of statins on plasma microRNA (miRNA) profile of individuals without coronary artery disease (CAD). Method: We measured the expressions of selected genes involved in AMPK pathway in THP-1 macrophages treated with simvastatin to assess dependence of their expression on cholesterol level. The Agilent's miRNA Microarray analyses were performed to compare plasma miRNA profile of individuals ( $\leq 30\%$  stenosis) treated with and without statin. MiRDB target prediction tool was utilized to identify miRNA target genes involved in AMPK pathway for differently expressed miRNAs. Expressions of target genes were analyzed by using real-time PCR. Results: Significantly decreased expression of 5 miRNAs (miR-625-5p, miR-550a-3-5p, miR-550b-2-5p, miR-550a-5p, miR-let-7d-5p) were observed in individuals receiving statins compared to non-statin group (fold change  $>1.5$ ,  $p < 0.05$ ). In addition, simvastatin increased the expression of AMPK  $\alpha 1$ , AMPK  $\alpha 2$ , AMPK  $\beta 1$ , AMPK  $\beta 2$  and SREBF2 in THP-1 macrophages. Using miRDB target prediction tool, we identified AMPK  $\alpha 1$  is a strong candidate (target score = 95) for miR-625-5p. Conclusion: Our results suggests that statin may downregulate the expression of miR-625-5p and induce AMPK $\alpha 1$  expression which in turn may activates cholesterol synthesis pathway. Apoptosis-associated miR-625 may be used as a novel biomarker for effectiveness of statins therapy in patients with atherosclerosis.

#### P05.53

#### From Facebook to gene: how social media helped to find a gene for a cardiac disease

W. S. Kerstjens-Frederikse<sup>1</sup>, B. Frentz<sup>1</sup>, H. G. Yntema<sup>2</sup>, T. Rinne<sup>2</sup>, I. van der Burgt<sup>2</sup>, P. A. van der Zwaag<sup>1</sup>, K. Löhrner<sup>1</sup>, B. Flapper<sup>3</sup>, C. M. A. van Ravenswaaij-Arts<sup>4</sup>;

<sup>1</sup>Department of Genetics, University of Groningen, University Medical Center, Groningen, Netherlands, <sup>2</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>3</sup>Department of Pediatrics, University of Groningen, University Medical Center, Groningen, Netherlands.

A patient support group, communicating with each other through Facebook, requested us to develop research on chromosome 6 anomalies. With the help of this patient support group, we recruited 253 patients with deletions or duplications of chromosome 6. We received detailed clinical information (including array reports) of 101 of these patients.

Genotype-phenotype comparison revealed a recurrent phenotype in seven patients with an overlapping 6q25.1 deletion. TAB2 was the most likely candidate gene in this region. Their phenotype (mitral valve defect and/or dilating cardiomyopathy (DCM), short stature, hyperlaxity and facial features, resembling Noonan syndrome) was so specific, that it could be matched to two unsolved familial cases without chromosome anomalies, known to our department. Exome sequencing and targeted sequencing of TAB2 was performed and mutations segregating with the phenotype were detected in both families. We concluded that mutations of TAB2 and deletions of chromosome 6q25.1 including TAB2 are associated with cardiac disease and a Noonan syndrome-like phenotype. We show that a Facebook based patient support group was able to participate in research, leading to the recognition of a disease gene and the associated phenotype.

#### Funding

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## P05.55

## Zebrafish model for MYBPC3 variants demonstrates pathogenicity

*M. Sabater Molina<sup>1</sup>, M. Anchelin<sup>2</sup>, M. Cayuela<sup>2</sup>, I. Pérez Sánchez<sup>1</sup>, E. García-Molina<sup>1</sup>, M. Olmo Conesa<sup>1</sup>, M. Oliva Sandoval<sup>3</sup>, J. Gimeno Blanes<sup>1</sup>;*

<sup>1</sup>Inherited Cardiac Disease Unit, University Hospital Virgen de la Arrixaca, Murcia, Spain, <sup>2</sup>University Hospital Virgen de la Arrixaca, Murcia, Spain, <sup>3</sup>Cardiology Department, University Hospital Virgen de la Arrixaca, Murcia, Spain.

**Introduction:** The majority of founder MYBPC3 mutations that cause hypertrophic cardiomyopathy (HCM) are truncation mutations, nevertheless few functional studies exist regarding the different factors that modify HCM phenotypes. To elucidate the underlying mechanisms in the phenotypic differences of HCM caused by MYBPC3 truncated mutation using a zebrafish model.

**Material and Methods:** We performed the functional study of two new founder mutations in MYBPC3 which cause HCM and origin truncated proteins of different lengths: variant A (p.Pro108Alafs\*9, -91%) and variant B (p.R891Afs\*160, -17%). We injected MYBPC3 variants A or B mRNA in zebrafish embryos at one cell stage to analyze the cardiac size and function.

**Results:** We created a viable zebrafish model of HCM with expression of the human variants A and B. Pathogenicity was suggested by changes in cardiac chambers. Ventricular size was increased when MYBPC3 variant A mRNA was injected. Similar results were found with variant B vs control; nevertheless the differences were not significant between variant A and B. These preliminary results could indicate that two variants have a dominant effect over phenotype expression of HCM, in contrast with others authors who suggest haploinsufficiency mechanisms due to a markedly lower protein expression of small truncated transcripts. More studies are needed to complete the characterization of this animal model and to explore future therapies.

	Control	Variant A	Variant B	p
End Systolic Ventricular Area (mm <sup>2</sup> )	7.43x10 <sup>-3</sup> (1.14x10 <sup>-3</sup> )	8.45 x10 <sup>-3</sup> (7.27 x10 <sup>-4</sup> )	8.75 x10 <sup>-3</sup> (1.55 x10 <sup>-3</sup> )	0.057
End Diastolic Ventricular Area (mm <sup>2</sup> )	1.03 x10 <sup>-2</sup> (1.66 x10 <sup>-3</sup> )	1.27 x10 <sup>-2</sup> (1.27 x10 <sup>-3</sup> )	1.27 x10 <sup>-2</sup> (1.43 x10 <sup>-3</sup> )	0.002
EF (%)	62 (7.9)	54 (6.2)	58 (13.4)	0.300
Shortening Fraction Area (%)	72 (0.06)	67 (0.05)	69 (0.10879)	0.298

Data are mean (sd). EF (%) from estimated end systolic/end diastolic volume.

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## P05.56

## Interactions of coagulation Factor II G20210A and Factor V Leiden with cardiovascular risk factors increase risk of myocardial infarction.

*J. A. Camilleri\*, F. Borg Carbott\*, R. Attard, P. Dingli, C. Doggen, K. Cassar, R. Farrugia, S. Bezzina Wettinger;*  
*University of Malta, Msida, Malta.*

**Introduction:** The effect of Coagulation Factor II G20210A (FIIG20210A) and Factor V Leiden (FVL) combined with cardiovascular risk factors on the risk of myocardial infarction (MI) was studied in a Maltese population.

**Methods:** Samples from the Maltese Acute Myocardial Infarction (MAMI) study including 423 cases with first MI and 465 controls were tested for the FVL and FIIG20210A mutations using PCR-RFLP with MnII and HindIII respectively. Interactions with lifestyle and metabolic risk factors were tested using data from questionnaires and medical history. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated to estimate the risk of MI overall and in subgroups.

**Results:** The estimated risk of MI is 1.6 (95%CI 0.64-3.9) for FVL and 1.5 (95%CI 0.76-2.96) for FIIG20210A. An association between FVL and MI was observed in smokers [OR 5.3 (95%CI 1.3-21.5)] but not in non-smokers [OR 0.95 (95%CI 0.24-3.84)]. FIIG20210A was associated with a risk of MI amongst research subjects with at least one metabolic risk factor (diabetes, hypertension or hypercholesterolaemia), [(OR 3.5 (95%CI 1.5-8.4)]. In individuals having at least one triggering factor (metabolic and/or smoking), a 4.4-fold (95%CI 1.3-14.8) and 3.6-fold risk (95%CI 1.5-8.6) were observed with FVL and FIIG20210A respectively.

**Conclusion:** FIIG20210A and FVL increase risk of MI in the presence of certain cardiovascular risk factors with which they interact synergistically. This may be the basis of contradictory results in the literature. These findings highlight the importance of prevention and treatment of these risk factors.

\*These authors contributed equally to the study.

## P05.57

## Genetic evaluation of next-generation sequencing results in dilated cardiomyopathies

*I. Boronova<sup>1</sup>, J. Bernasovska<sup>1</sup>, J. Kmec<sup>2</sup>, I. Bernasovsky<sup>3</sup>, V. Szabadosova<sup>1</sup>, P. Ferenc<sup>1</sup>, E. Petrejcikova<sup>1</sup>;*

<sup>1</sup>Department of Biology, Faculty of Humanities and Natural Sciences, University of Presov, Presov, Slovakia, <sup>2</sup>Cardiocentre, Faculty Hospital of J.A.Rayman, Presov, Slovakia, <sup>3</sup>Institute of Roma Studies, University of Presov, Presov, Slovakia.

Dilated cardiomyopathy (DCM) is a genetically heterogeneous disease, known to be one of the leading causes of heart failure and sudden cardiac death. Due to the large number of causative genes and high rate of private mutations, mutational screening requires the use of extremely sensitive and specific detection methods. Recent technical advances by introduction of next generation sequencing (NGS) technologies provide new possibilities of dilated cardiomyopathies detection. The aim of our study was evaluation of genetic profile of next-generation sequencing results in Slovak patients with dilated cardiomyopathy. In the cohort of 19 unrelated patients with clinical diagnosis of dilated cardiomyopathy (n=19; 12 male, 7 female, age range 29-71 years) H558R mutations (exon 12) in SCN5A gene in heterozygous and homozygous form were detected. Altogether, 105 376 sequence variants were identified, 94% were single nucleotide sequence variants, 6% of sequence variants represented insertions and deletions. Among the 103 non-synonymous variants three sequence variants (rs34580776, rs41277370, rs942077) where predicted to be „damaging“ associated with cardiomyopathy (SIFT score 0-0.5). NGS analyses revealed also the presence of novel mutation of CANX gene. The results of our study reflect necessity of evaluation of genomic variants in the genes involved in cardiomyopathies as a part of NGS genetic testing. NGS analyses identifies novel DNA sequence variants for which pathogenicity is unresolved.

**Key words:** next generation sequencing, mutations, dilated cardiomyopathy  
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## P05.58

## The necessity of original NGS data reanalysis in the diagnosis of inherited cardiac diseases with hindsight of 2 years.

*J. Kadlecová<sup>1</sup>, A. Veverková<sup>2</sup>, D. N. Grochová<sup>1</sup>, M. Hiemerová<sup>1</sup>, M. Matyášová<sup>1</sup>, I. Grochová<sup>1,3,4</sup>, L. Skutková<sup>4</sup>, P. Vlašín<sup>1,3</sup>;*

<sup>1</sup>Cytogenetic laboratory Brno, s.r.o., Brno, Czech Republic, <sup>2</sup>Masaryk University Brno, Faculty of Science, Brno, Czech Republic, <sup>3</sup>Center of prenatal diagnosis, Brno, Czech Republic, <sup>4</sup>International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic.

Next generation sequencing, also called massively parallel sequencing (MPS), currently is the fastest growing method in the field of molecular genetics. This new technology enables diagnostics of genetically heterogeneous diseases with a significant phenotypic and genetic overlap. On the basis of precise molecular genetic diagnosis it is possible to clarify the diagnosis in patients with cardiac disease and especially to identify pre-symptomatic individuals in the family for purposes of monitoring or treatment. Here we present our diagnostic NGS workflow for the analysis of 46 genes involved in pathogenesis of the inherited cardiomyopathy using TrueSight Enrichment technology (Illumina) and for the 51 genes described in association with arrhythmia disorders using PED Mast Plus technology (Multiplicom) followed by sequencing on the MiSeq (Illumina). This study comprised group of 130 unrelated patients with DCM, ARVC, ventricular fibrillation and arrhythmias.

In this study we reanalysed variants detected in our group of patients with a hindsight of 2 years. Based on different databases (dbSNP, ClinVar etc.) as well as co-segregation analysis in pedigrees we revealed that several variant originally classified as pathogenic or potentially pathogenic were false-positive and are newly classified as variants with unknown clinical significance or benign. We will demonstrate this discrepancy on several selected cases and family pedigrees.

Our findings point to the need of periodical reassessment of described variants because their interpretation is constantly changing. Moreover, tight cooperation, clinical and NGS data sharing between investigators focused on the same diagnostic is extremely important to clarify and interpret detected variants.

**P05.59****A genome-wide association study in ethnic Arabs reveals novel susceptibility loci for obesity**

*N. Dzimir<sup>1</sup>, S. M. Wakil<sup>1</sup>, R. Ram<sup>2</sup>, N. P. Muiya<sup>1</sup>, O. Alboudari<sup>1</sup>, E. Andres<sup>1</sup>, N. Mazhar<sup>1</sup>, S. Hagos<sup>1</sup>, B. F. Meyer<sup>1</sup>, G. Morahan<sup>2</sup>, M. Alshahidi<sup>1</sup>*

<sup>1</sup>King Faisal Spec. Hospital and Research Centre, Riyadh, Saudi Arabia, <sup>2</sup>University of Western Australia, Perth, Australia.

**Introduction:** Obesity (OBS) constitutes a major predisposing factor for many cardiovascular diseases. Genome-wide association approach continues to reveal association of gene polymorphism with OBS in different population. Such studies have not been reported for ethnic Arabs.

**Materials:** In this study, we performed a genome-wide association study using the Affymetrix High Density Axiom platform GWAS for OBS incidence in 5,016 Saudis of Arab ancestry (3013 controls, 2003 cases).

**Results:** In the discovery set, we identified four susceptibility SNPs for OBS: the rs564702 (OR=1.31; P=1.85x10<sup>-8</sup>) residing on the KCNMB2 gene, rs16891982 (OR=1.56; P=4.31x10<sup>-8</sup>) on the SLC45A2 gene, rs2523946 (OR = 1.30; P=2.72x10<sup>-8</sup>) on the MICD and rs7180536 (OR=1.29; P=4.82x10<sup>-8</sup>) on the SQRDL gene that demonstrated genome-wide significance levels. Furthermore, two variants, the rs7551194 (OR=1.36; p=7.22x10<sup>-7</sup>) on POU3F1 and rs2171693 (OR=1.39; P=1.08x10<sup>-7</sup>) on the PBX1 gene both located on chr1, were also strongly linked to obesity. Besides, three other variants, rs10810271 (OR=1.38; p=6.02 x 10<sup>-6</sup>) on the FREM1, rs7135639 (OR=1.25; p=5.19x10<sup>-6</sup>) on the MED13L and rs7890737 (OR=1.30; p=6.92x10<sup>-6</sup>) on the GPM6B as well as two protective variants, the rs17118166 (OR=0.71; P=3.80x10<sup>-6</sup>) on SGCD and rs35488337 (OR=0.74; p=3.88x10<sup>-6</sup>) on ETS1 gene showed borderline association with disease. These variants retained their associations in the replication set, and several haplotypes were similarly implicated in the disease. We also replicated 3 SNPs on chromosome 9 that have been associated with OBS in different populations.

**Conclusion:** These findings provide new insights into genetic pathways contributing to the susceptibility for OBS in ethnic Arabs.

**P05.60****Therapy dependent role of an Oct1 polymorphism in cardiovascular death in patients with type 2 diabetes**

*N. Schweighofer<sup>1</sup>, B. Genser<sup>2,3</sup>, W. März<sup>4</sup>, M. E. Kleber<sup>5</sup>, O. Trummer<sup>1</sup>, T. R. Pieber<sup>4,6</sup>, B. Obermayer-Pietsch<sup>1,6</sup>*

<sup>1</sup>Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University, Graz, Austria, <sup>2</sup>BG Statistical Consulting, Vienna, Austria, <sup>3</sup>Institute of Public Health, Social and Preventive Medicine, Medical Faculty of Mannheim, University of Heidelberg, Mannheim, Germany, <sup>4</sup>Synlab Center of Laboratory Diagnostics, Heidelberg, Germany, <sup>5</sup>Department of Molecular Evolution and Genomics, University of Heidelberg, Heidelberg, Germany, <sup>6</sup>CBmed, Center for Biomarker Research in Medicine, Graz, Austria.

**AIM:** Type 2 diabetes individuals have high risk for microvascular complications and cardiovascular incidents are increased two- to three-fold compared to non-diabetics. Various substrates of organic cation transporter 1 are associated with cardiovascular biomarkers. We investigated, whether the Oct1 polymorphism rs2297374 is associated with cardiovascular biomarkers and with the risk of cardiovascular death and if the associations found are influenced by antidiabetic therapy.

**METHODS:** The data from the LURIC study, a prospective cohort study of Caucasian individuals scheduled for coronary angiography, were used. We identified 1208 type 2 diabetics, whereof 72 use metformin and 1135 are not on metformin therapy. Cardiovascular mortality was assessed in non-diabetics, type 2 diabetics and metformin and non-metformin users with different Oct1 rs2297374 genotypes using Cox proportional hazard models and associations with cardiovascular biomarkers were investigated.

**RESULTS:** Cardiovascular mortality risk was for each minor allele copy HR 0.83 95% CI 0.70, 0.98 in patients with T2DM and HR 0.82 CI 0.69-0.97 in non-metformin users. In these 2 patients groups the variants of rs2297374 were significantly associated with HDL and total cholesterol levels. No differences in cardiovascular mortality and no associations with the investigated biomarkers were seen in non-diabetics and type 2 diabetics on metformin therapy.

**CONCLUSION:** The minor C allele of rs2297374 in the Oct1 gene is associated with decreased cardiovascular mortality in non-metformin users eventually due to its influence on HDL and total cholesterol levels.

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**P05.61****Genome-wide multi-phenotype and eQTL analyses provide novel insights into omega fatty acid metabolism**

*M. Kaakinen<sup>1</sup>, A. Claringbould<sup>1,2</sup>, F. Hagenbeek<sup>1,3,4</sup>, R. Mägi<sup>5</sup>, P. Soininen<sup>6</sup>, M. Järvelin<sup>1,7</sup>, BIOS Consortium, A. P. Morris<sup>8</sup>, I. Prokopenko<sup>1</sup>*

<sup>1</sup>Imperial College London, London, United Kingdom, <sup>2</sup>University Medical Centre Groningen, Groningen, Netherlands, <sup>3</sup>VU University Amsterdam, Amsterdam, Netherlands, <sup>4</sup>EMGO+ Institute for Health and Care Research, Amsterdam, Netherlands, <sup>5</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>6</sup>University of Eastern Finland, Kuopio, Finland, <sup>7</sup>University of Oulu, Oulu, Finland, <sup>8</sup>University of Liverpool, Liverpool, United Kingdom.

**Introduction:** There is evidence for favourable effects of diets rich in omega-3 fatty acids (FAs) on cardiovascular disease. However, little is known on the genetic contribution to FA levels, since most studies have focused on serum lipid concentrations typically used in clinical practice.

**Materials and Methods:** We undertook common and rare variant genome-wide multi-phenotype analyses (MPA) of FA levels using nuclear magnetic resonance-based measures of FAs and 1000 Genomes imputed data from the Northern Finland Birth Cohorts 1966 (N=4949) and 1986 (N=3055). To avoid multicollinearity issues, we selected four FAs for MPA: omega-3, -6, -7/9 and other polyunsaturated FAs. Expression quantitative locus (eQTL) analysis was performed on the Dutch BIOS consortium RNA-seq data (N=2116).

**Results:** The common variant meta-analysis detected 10 signals associated with FAs ( $P < 5 \times 10^{-8}$ ), including a novel FA locus at *MACROD1* (rs1006207). Variation in the other identified loci *PCSK9*, *GCKR*, *FADS1*, *ZNF259*, *LIPC*, *PDXDC1*, *PBX4*, *APOE* and *ADAMTS3* has previously been associated with triglycerides and cholesterol levels, but not specifically with FAs. *FADS1* was also identified in the rare variant MPA. The eQTL analysis indicated eight out of 10 MPA-identified signals with significant cis-eQTL effects on gene expression levels. The novel *MACROD1* variant was a cis-eQTL for *PRDX5* expression, suggesting a role in protection against oxidative stress.

**Conclusions:** With a combination of refined lipid measures, MPA and eQTL analysis we have identified genetic loci and differentially expressed genes involved in FA metabolism, providing novel clues for the complex relationship between lipids and cardiovascular disease.

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**P05.62****The effects of diabetes mellitus and/or genetic polymorphisms on the formation of the peripheral arterial diseases**

*Z. Yalim<sup>1</sup>, S. Tutgun Onrat<sup>2</sup>, O. Akci<sup>1</sup>, G. Ozkececi<sup>1</sup>, M. Aldemir<sup>3</sup>, A. Avsar<sup>1</sup>, I. Dogan<sup>4</sup>, E. Onrat<sup>1</sup>*

<sup>1</sup>Afyon Kocatepe University, Faculty of Medicine, Department of Cardiology, Afyonkarahisar, Turkey, <sup>2</sup>Afyon Kocatepe University, Faculty of Medicine, Department of Medical Genetics, Afyonkarahisar, Turkey, <sup>3</sup>Afyon Kocatepe University, Faculty of Medicine, Department of Cardiovascular Surgery, Afyonkarahisar, Turkey, <sup>4</sup>Afyon Kocatepe University, Faculty of Medicine, Department of Biostatistics, Afyonkarahisar, Turkey.

**Introduction:** Peripheral arterial disease(PAD) is a condition that is caused by narrowing of limb arteries due to atherosclerosis. Femoral, popliteal and tibial arteries are more affected in patients with diabetes mellitus(DM). In this study, we investigated the effects of diabetes mellitus and genetic background, solely and together in the pathogenesis of PAD.

**Materials and Methods:** In our study we planned to evaluate the effects of Factor V LEIDEN, Factor V H1299R, Prothrombin G20210A, Factor XIII V34L, B-Fibrinogen -455 G>A, PAI-1 4G/5G, HPA1 , MTHFR C677T, MTHFR A1298C, ACE I/D, APO B R3500Q, and APO E polymorphisms using a VienLab CVD StripAssay. Patients were placed into four groups: 50 patients with DM and PAD, 50 patients with PAD and without DM, 30 patients with DM and without PAD and 30 controls.

**Results:** In the formation of PAD regardless of the presence of diabetes, MTHFR A1298C and PAI 4G/5G homozygous mutation seems to be determinant ( $p < 0.05$  for both). In the presence of DM, PAI 4G/5G homozygous mutation determined the formation of PAD ( $p < 0.05$ ). In regression analysis, PAI-1 4G/5G gene homozygous mutation was 17,1 times more risky, ( $p < 0.008$ ) 95% CI(2,113-138,660), MTHFR A1298C homozygous mutation was 316,6 times more risky ( $p < 0.001$ ) 95% CI(10,763-9315,342) the possibility of diabetes mellitus with peripheral artery diseases.

**Conclusions:** It seems to be that, in the presence of DM, PAI 4G/5G homozygous mutation is powerfull predictor to determine the formation of PAD. In the regardless of DM, MTHFR A1298C and PAI 4G/5G homozygous mutation are also predictor in the formation of PAD.

**P05.63****New insights in Pseudohypoaldosteronism type II**

G. J. Voortman, A. J. M. Verkerk, E. J. G. Sijbrands, J. Versmissen, A. H. van den Meiracker, E. C. H. Friesema;  
Erasmus University Medical Center, Rotterdam, Netherlands.

Pseudohypoaldosteronism type II (PHAI), also known as Gordon's syndrome or familial hyperkalemic hypertension (OMIM 145260), is a rare mono-genetic disorder mainly characterized by arterial hypertension and hyperkalemia. So far, 4 genes have been identified to be mutated in PHAI patients, either WNK1, WNK4, KLHL3 or CUL3. All patients can be effectively treated by thiazide diuretics, compatible with over-activity of the thiazide-sensitive Na<sup>+</sup>-Cl<sup>-</sup> cotransporter in the kidney.

A 60 years old female patient had been diagnosed with hyperkalemia e.c.i. > 10 years. Metabolic acidosis and hypertension were not present. Plasma potassium level was 6.3 mmol/L (normal range 3.5-5.1 mmol/L) as well as a mild hyperchloremia with low plasma levels of aldosterone and low-normal plasma levels of renin.

Sanger sequencing revealed a novel heterozygote G>A mutation in exon 11 of KLHL3, leading to an amino acid change (V434M). This mutation was adjacent to already known disease-causing mutations. Prediction models indicate that this V434M has a deleterious effect on KLHL3 function. No additional mutations were found in WNK1, WNK4, and CUL3. The patient was treated with hydrochlorothiazide, which resulted in normalization of potassium plasma levels as well as in lowering of her normal blood pressure. Genetic analysis of her two daughters, revealing the same hyperkalemia (6.3 and 5.9 mmol/L, respectively) without hypertension, showed that both carry the same heterozygote mutation in KLHL3. They were also treated with hydrochlorothiazide.

The genetic analysis of KLHL3 in this family revealed that the diagnosis of PHAI is not always the obligatory combination of hyperkalemia plus hypertension.

**P05.64****Whole genome sequencing and deep phenotyping identifies rare protein-coding and non-coding variants in a large cohort of unrelated idiopathic and heritable pulmonary arterial hypertension cases**

S. Gräf<sup>1</sup>, M. Bleda<sup>1</sup>, C. Hadinapola<sup>1</sup>, M. Haimel<sup>1</sup>, H. J. Bogaard<sup>2</sup>, G. Coghlan<sup>3</sup>, P. A. Corris<sup>4</sup>, S. Gibbs<sup>5</sup>, D. G. Kieb<sup>6</sup>, A. Lawrie<sup>7</sup>, R. D. Machado<sup>8</sup>, A. J. Peacock<sup>9</sup>, J. Pepke-Zaba<sup>10</sup>, M. Toshner<sup>10</sup>, R. C. Trembath<sup>11</sup>, A. Vonk Noordegraaf<sup>12</sup>, J. Wharton<sup>12</sup>, M. R. Wilkins<sup>12</sup>, S. J. Wort<sup>13</sup>, N. W. Morrell<sup>14</sup>, on behalf of the NIH/NR BioResource Rare Diseases (BRIDGE) PAH Consortium, and the UK National PAH Cohort Study Consortium;

<sup>1</sup>University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>VU University Medical Centre Amsterdam, Amsterdam, Netherlands, <sup>3</sup>Royal Free Hospital, London, United Kingdom,

<sup>4</sup>Freeman Hospital, Newcastle, United Kingdom, <sup>5</sup>Hammersmith Hospital, London, United Kingdom, <sup>6</sup>Royal Hallamshire Hospital, Sheffield, United Kingdom, <sup>7</sup>University of Sheffield, Sheffield, United Kingdom, <sup>8</sup>University of Lincoln, Lincoln, United Kingdom, <sup>9</sup>Golden Jubilee National Hospital, Glasgow, United Kingdom, <sup>10</sup>Papworth Hospital, Papworth, United Kingdom, <sup>11</sup>King's College, London, United Kingdom, <sup>12</sup>Imperial College, London, United Kingdom, <sup>13</sup>Royal Brompton Hospital, London, United Kingdom.

**Introduction:** Despite the identification of rare causal variation in several genes, most notably BMPR2, a large proportion of cases of heritable (~20%) and idiopathic pulmonary arterial hypertension (PAH) (~80%) still remain unexplained at the molecular level.

**Methods:** As part of the UK 10,000 Genomes BRIDGE project the whole genomes of 1,250 unrelated individuals diagnosed with idiopathic and heritable PAH are being sequenced and extensively phenotyped. Stringent filtering based on MAF < 1/10,000 in both BRIDGE and ExAC and deleteriousness and conservation scores are used to prioritise pathogenic variants, coupled with phenotypic information.

**Results:** To date, 5844 whole genomes of rare disease patients have been sequenced including 625 PAH cases. 107 PAH subjects (17%) possess clearly or likely pathogenic mutations in one of the known PAH genes. The majority of loss-of-function and likely pathogenic missense mutations have been identified in BMPR2 (73 PAH cases [12%]). A further 14 subjects had (micro-)deletions of 5kb to 3.8Mb affecting either the entire BMPR2 locus or one or more exons within the gene. Additional variants in the promoter and other non-coding regions of BMPR2 are undergoing functional investigation. We also identified individuals with homozygous (n=6) and potential compound heterozygous (n=6) mutations in EIF2AK4 (2%).

**Conclusions:** Of the known PAH genes, BMPR2 mutations are the most common. It is likely that non-coding variation around known PAH genes, in particular BMPR2, will explain further PAH cases. The genetics underlying PAH appear complex and a large sample size is necessary to test for novel rare sequence variation association.

**P05.65****Novel SMAD2 mutations in two families with LDS-like syndromic presentations of aortic aneurysm**

E. Cannaeerts<sup>1</sup>, M. Kempers<sup>2</sup>, J. Richer<sup>3</sup>, L. Beauchesne<sup>4</sup>, A. Verstraeten<sup>1</sup>, N. Peeters<sup>1</sup>, B. Loey<sup>1</sup>, L. Van Laer<sup>1</sup>;

<sup>1</sup>Center of Medical Genetics, Faculty of Medicine and Health Sciences, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium, <sup>2</sup>Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands,

<sup>3</sup>Department of Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada, <sup>4</sup>Division of Cardiology, University of Ottawa Heart Institute, Ottawa, ON, Canada.

Aortic aneurysms/dissections are common manifestations in hereditary connective tissue disorders, including Marfan syndrome (MFS) and Loey-Dietz syndrome (LDS). Exploration of underlying pathomechanisms has revealed prominent dysregulation of the transforming growth factor (TGF)- $\beta$  signaling pathway. Most recently, mutations in SMAD2, encoding a key transcriptional regulator of TGF- $\beta$  signaling, have been shown to cause aortic aneurysmal disease. Here, we expand the phenotypical spectrum associated with SMAD2 mutations in two families presenting with syndromic aortopathy.

The application of a custom targeted resequencing panel, including SMAD2, led to the identification of two novel missense mutations, both affecting highly conserved amino acids in the functionally important DNA binding MH2 domain of SMAD2.

The first mutation (p.Asn318Lys) was found in a 63-years old female proband presenting with an LDS phenotype: facial features (high arched palate, broad uvula, downslanting palpebral fissures), skeletal abnormalities (arachnodactyly, pectus excavatum and scoliosis surgeries at ages 11 and 36 years), aortic root replacement (age 53 years). Segregation analysis revealed the presence of the SMAD2 mutation in the daughter who presents with marfanoid features. The second mutation carrier (p.Ser397Tyr) was diagnosed with LDS. Cardiovascular symptoms include aortic root dilatation and marked tortuosity of the neck vessels, no major skeletal abnormalities were noted. We confirmed presence of the mutation in a daughter with hypertelorism and arterial tortuosity and mother with hypertelorism and normal echocardiography.

In conclusion, SMAD2 mutations are causal in patients with LDS and MFS features. This gene should be included in the expanding list of candidate genes causing these conditions.

**P05.66****Search for somatic mitochondrial DNA mutations in carotid atherosclerotic plaques by massive parallel sequencing**

M. V. Golubenko<sup>1,2,3</sup>, N. P. Babushkina<sup>1</sup>, M. S. Nazarenko<sup>1,3</sup>, A. V. Markov<sup>1,3</sup>, S. V. Buikin<sup>1</sup>, A. A. Sleptsov<sup>1,3</sup>, O. L. Barbarash<sup>2</sup>, V. P. Puzyrev<sup>1,3</sup>;

<sup>1</sup>Institute for Medical Genetics, Tomsk, Russian Federation, <sup>2</sup>Institute for complex issues of cardiovascular diseases, Kemerovo, Russian Federation, <sup>3</sup>Tomsk State University, Tomsk, Russian Federation.

**Introduction:** Atherosclerosis is accompanied by increased oxidative stress. MtDNA is exposed to oxidative damage, since it is located near the ROS production sites. To describe possible somatic mtDNA mutations arising during atherogenesis, we have compared heteroplasmic mtDNA variations in the carotid plaques and blood using massive parallel sequencing.

**Materials and methods:** Ten paired samples of DNA extracted from the ablated carotid plaques and from the blood of the patients, were analyzed. MtDNA was amplified in two overlapped PCR-products, sequenced and analyzed on the MiSeq platform, using 1% threshold for variant calling. Single nucleotide indels were not considered if detected in more than two individuals. **Results:** The mean base coverage across the mitochondrial genome was about 8000. All individuals had heteroplasmic positions at the minimum 1% level (average 3.2 positions per plaque sample and 3.1 positions per blood sample). Altogether, 50 heteroplasmic positions were revealed, 12 of them were common for the blood and plaque samples, and only 1 position was found to be heteroplasmic in two individuals. The heteroplasmic positions were concentrated in the control region (30%), and in protein-coding genes (54%). Transition to transversion ratio was elevated (9:1), as well as nonsynonymous mutations (73% of all protein-coding heteroplasmies, including 2 frameshifts), comparing to population polymorphisms.

**Conclusions:** There is considerable heteroplasmic mtDNA variation in atherosclerosis, with no significant differences between blood and carotid plaques. The somatic mtDNA variation characteristics differ from those of inherited polymorphisms.

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## P05.70

### The involvement of ER-retention and quality control in the cellular mechanisms underlying hereditary hemorrhagic telangiectasia and familial pulmonary arterial hypertension caused by mutations in TGF- $\beta$ growth factor receptors

B. R. Ali, P. Kizhakkedath, A. John, L. Al-Gazali;  
United Arab Emirates University, Al-Ain, United Arab Emirates.

**Introduction:** The transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily signaling plays critical roles in the regulation of cellular growth, differentiation and development. Mutations in the plasma membrane components of the TGF- $\beta$  signaling pathways including mutations in BMPR2, ENDOGLIN and ALK1 genes have been shown to cause pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia types 1 (HHT1) and 2 (HHT2), respectively. However, the underlying cellular mechanisms of the majority of the reported mutations are unknown.

**Materials and Methods:** The C-terminally tagged cDNAs of BMPR2, ENDOGLIN and ALK1 in mammalian expression vectors were used as templates to generate the required missense disease-causing mutations by QuikChange site-directed mutagenesis. The constructs were transfected into HeLa and HEK293 cells and the expressed proteins were analyzed biochemically and by confocal microscopy.

**Results:** Ten of the examined eighteen missense mutants in BMPR2 have shown aberrant subcellular trafficking with the extracellular domain mutants predominantly retained within the lumen of the ER. Similarly, the majority of the analyzed missense mutants in ALK1 and ENDOGLIN exhibited aberrant trafficking and intracellular retention by the ER quality control machinery. In addition, we found that the plasma membrane trafficking of these mutants was amenable to correction by chemical chaperones.

**Conclusions:** ER retention of TGF- $\beta$  mutant proteins is a major cellular mechanism underlying pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia. Manipulation of this ER retention is a potential target for therapeutic intervention in these diseases.

## P05.71

### Targeted Next-Generation Sequencing Helps to Unravel the Genetic Heterogeneity of Hypertrophic Cardiomyopathy

M. Cecconi<sup>1</sup>, M. I. Parodi<sup>1</sup>, F. Formisano<sup>2</sup>, P. Spirito<sup>2</sup>, C. Autore<sup>3</sup>, M. B. Musumeci<sup>3</sup>, S. Favale<sup>4</sup>, C. Forleo<sup>4</sup>, C. Rapezzi<sup>5</sup>, E. Biagini<sup>5</sup>, S. Davi<sup>1</sup>, E. Canepa<sup>1</sup>, L. Pennese<sup>1</sup>, M. Castagnetta<sup>1</sup>, D. Degiorgio<sup>1,6</sup>, D. A. Coville<sup>1</sup>;

<sup>1</sup>Laboratory of Human Genetics, E.O. Ospedali Galliera, Genoa, Italy, <sup>2</sup>Cardiology Unit, E.O. Ospedali Galliera, Genoa, Italy, <sup>3</sup>Cardiology Unit, St. Andrea Hospital, Sapienza University, Rome, Italy, <sup>4</sup>Cardiology Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy, <sup>5</sup>Cardiology Unit, Policlinico S. Orsola-Malpighi, University of Bologna, Bologna, Italy, <sup>6</sup>Department of Experimental Medicine, University of Genoa, Genoa, Italy.

Hypertrophic cardiomyopathy (HCM) is caused mostly by mutations in MYH7 and MYBPC3 genes; less frequently mutations in other genes have been reported. The aim of this study is to evaluate a target-Next Generation Sequencing (NGS) panel, which includes sarcomeric genes not only encoding thick/intermediate and thin myofilament (TTm) proteins, but also other sarcomeric genes and some causative genes of HCM-phenotype. A Ion AmpliSeq™ Custom Panel for the enrichment of 19 genes, 9 of which not encoding TTm proteins, was established. By using Ion Personal Genome Machine, we studied: (i) a training set (n. 73 DNA-samples) already genotyped to optimize the NGS strategy; (ii) a discovery set (n. 19 DNA-samples). In the training set we identified 71 out of 72 expected mutations and 15 additional mutations, 10 of which in genes not encoding TTm proteins; of them, 4 are identified in MYOM1 gene. In the discovery set we identified 20 mutations: the diagnostic yield was increased of about 20% and, among the mutations not belonging to genes encoding TTm proteins, we identified mutant alleles of MYOZ2 (one), MYOM1 (two) and LAMP2 (one) genes. Our combined targeted NGS-Sanger sequencing-based strategy allowed: a) the molecular diagnosis of HCM with greater efficiency than using the conventional (Sanger) sequencing alone; b) the identification of less frequently mutant alleles associated to HCM like MYOM1 and MYOZ2 genes (Siebert et al., 2011; Osio et al., 2007). Mutant alleles identification in genes not encoding sarcomeric TTm proteins may better contribute to explain the genetic heterogeneity of HCM.

## P05.72

### Study of the contribution of copy number variation to the pathogenesis of bicuspid aortic valve associated aortopathy

I. Luyckx<sup>1</sup>, E. Reyniers<sup>1</sup>, E. Dekeyser<sup>1</sup>, K. Vanderstraeten<sup>1</sup>, G. Vandeweyer<sup>1</sup>, F. Wünnemann<sup>2</sup>, C. Preuss<sup>2</sup>, P. Eriksson<sup>3</sup>, S. A. Mohamed<sup>4</sup>, G. Andelfinger<sup>2</sup>, A. Verstraeten<sup>1</sup>, L. Van Laer<sup>1</sup>, B. L. Loey<sup>1,5</sup>, MIBAVA Leducq consortium;

<sup>1</sup>Center for Medical Genetics, Edegem, Belgium, <sup>2</sup>Cardiovascular Genetics, Department of Pediatrics, Centre Hospitalier Universitaire Saint-Justine Research Centre, Université de Montréal, Montreal, QC, Canada, <sup>3</sup>Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden, <sup>4</sup>Department of Cardiac and Thoracic Vascular Surgery, University Clinic of Schleswig-Holstein, Luebeck, Germany, <sup>5</sup>Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

Affecting 1-2% of the population, bicuspid aortic valve (BAV) is the most common congenital heart malformation. Although it is usually asymptomatic, 10-20% of individuals with BAV develop life-threatening thoracic aortic aneurysms (TAA). Several studies have indicated that up to 10% of left-sided heart defects are caused by deleterious copy number variations (CNVs). Hence, we hypothesize that CNVs may also contribute to the pathogenesis of BAV/TAA.

CNV analysis was performed in 100 unrelated BAV/TAA patients using the CytoSNP12.2 array, followed by data analysis with the CNV-WebStore tool. CNVs affecting protein-coding genes with a potential role in the cardiovascular system and with a low frequency in healthy individuals (MAF<0.5%) were manually prioritised for validation with MLPA/qPCR/MAQ.

Each BAV/TAA patient carried approximately 14 CNVs, which is comparable to what is observed in healthy individuals. After filtering based on gene content, 15 candidates remained, of which six could be validated. These CNVs did not encompass any known BAV/TAA genes and segregation analysis did not support fully-penetrant pathogenicity. According to DECIPHER, CNVs overlapping with candidate CNVs were also identified in controls or in highly dissimilar diseases without cardiovascular involvement, further demonstrating the unlikelihood that these CNVs directly cause BAV/TAA.

Our results show that this BAV/TAA cohort is not enriched for deleterious CNVs causing BAV/TAA. This finding is in contrast to prior studies of other left-sided heart defects, which identified CNVs as a major causative factor. However, we cannot exclude the possibility that certain CNVs contribute as modifiers, creating a sensitised background for BAV/TAA.

## P05.73

### A common variant association study for type 2 diabetes mellitus in ethnic Saudi Arabs

D. Bakheet<sup>1</sup>, N. P. Muiya<sup>1</sup>, S. M. Wakil<sup>1</sup>, R. Ram<sup>2</sup>, B. F. Meyer<sup>1</sup>, G. Morahan<sup>2</sup>, N. Dzimiri<sup>1</sup>;

<sup>1</sup>King Faisal Spec. Hospital and Research Centre, Riyadh, Saudi Arabia, <sup>2</sup>Western Australian Institute for Medical Research, University of Western Australia, Australia, Perth, Australia.

**Introduction:** Type 2 diabetes mellitus (T2D) is a debilitating disease that contributes to the manifestation of complex disease, such as coronary heart disease, with a strong genetic component. However the genetic basis for the disease is not fully understood yet.

**Materials and Method:** In the present study, we performed a genome-wide association study using the Affymetrix High Density Axiom platform for the disease in the ethnic Saudi Arab population (cases versus).

**Results:** In all, in the discovery study, we identified nine variants that displayed significant ( $P \leq 10^{-7}$ ) or suggestive ( $P \leq 10^{-6}$ ) association with the disease. The lead variant was the rs4506565 [1.29(1.19-1.40);  $p=8.78E-10$ ] on chr10 in the TCF7L2 gene. Another variant rs16976967 [1.22(1.12-1.33);  $p=4.17E-06$ ] on chr15 in the MNS1 gene showed suggestive association with disease. On the other hand seven other variants showed weak protective properties against the disease. These included the rs10789192 [0.83(0.76-0.9);  $p=4.52E-06$ ] on chr1 in the PDE4B gene, rs681828 [0.82(0.76-0.88);  $p=5.44E-07$ ] on chr1 in the KCNK1 gene, rs13411975 [0.84(0.77-0.92);  $p=8.76E-06$ ] on chr2 in the SNTG2 gene, rs9824601 [0.82(0.76-0.88);  $p=1.39E-07$ ] on chr3 in the MRPL3 gene, rs4352831 [0.88(0.82-0.95);  $p=6.48E-06$ ] on chr8 in the ADCY8 gene, rs150524 [0.82(0.76-0.89);  $p=1.47E-06$ ] on chr17 in the CCL1 gene and the intergenic rs9948423 [0.7(0.6-0.81);  $p=2.15E-06$ ] on chr18. Interestingly, we were able to validate the associations of these variant with disease in a separate cohort.

**Conclusions:** The study revealed several variants throughout the genome that conferred risk for type 2 diabetes mellitus. Our validation experiment suggests a strong genetic link to the disease in ethnic Arabs.

**P05.74****Next generation sequencing to unravel the genetic architecture of *KNG1* and *F11* loci**

L. Martin-Fernandez<sup>1</sup>, G. Gavidia<sup>1</sup>, I. Corrales<sup>2</sup>, H. Brunel<sup>1</sup>, L. Ramírez<sup>2</sup>, J. C. Souto<sup>3</sup>, F. Vidal<sup>1</sup>, J. M. Soria<sup>1</sup>;

<sup>1</sup>Unit of Genomics of Complex Diseases, Biomedical Research Institute Sant Pau (IIB-Sant Pau), Barcelona, Spain, <sup>2</sup>Unit of Molecular Diagnosis and Therapy, Blood and Tissue Bank, Barcelona, Spain, <sup>3</sup>Unit of Hemostasis and Thrombosis, Department of Hematology, Barcelona, Spain.

**Introduction:** Factor XI (FXI) level is an intermediate phenotype of venous thromboembolism -- a complex disease with a high heritability (~60%). The Genome Wide Association Study (GWAS) performed in the GAIT-1 (Genetic Analysis of Idiopathic Thrombophilia) Project revealed significant associations between the FXI levels and SNPs in *KNG1* and *F11* genes. Our aim was to identify the genetic variability of *KNG1* and *F11* that could explain such association.

**Materials and Methods:** The *KNG1* and *F11* were completely sequenced (exons, introns and promoters) in 110 unrelated individuals from the GAIT-2 Project using Next generation sequencing (NGS) Illumina MiSeq Platform. Association analyses were performed with FXI levels using the PLINK and SKAT package. Also we identified by data filtering and *in silico* predictions the putative causal mutations.

**Results:** A total of 762 genetic variants were detected. Several significant nominal associations were identified between common variants and clustered low frequency variants in *KNG1* and *F11* with FXI levels. Among these associations, the missense rs710446 was significant after the permutation test. In addition, two putative causal mutations were related with high and low FXI levels.

**Conclusions:** Using NGS we investigated the genetic architecture of the *F11* and *KNG1* loci. This information contributes to the identification of the "missing heritability" of this key intermediate phenotype. Concretely, we identified putative rare mutations, common variants and clustered low frequency variants associated with FXI levels that may contribute to the risk of venous thromboembolism.

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**P06 Metabolic and mitochondrial disorders****P06.01****Whole-exome sequencing as diagnostic tool in a quartet family with Abetalipoproteinemia**

A. Sanchis-Juan<sup>1</sup>, D. Perez-Gil<sup>1</sup>, C. Martinez-Costa<sup>2</sup>, V. Martínez-Barquero<sup>1</sup>, V. Gonzalez-Albert<sup>1</sup>, P. Marín-García<sup>1</sup>, F. J. Chaves<sup>1,3</sup>, A. García-García<sup>1,3</sup>;

<sup>1</sup>Genotyping and Genetic Diagnosis Unit, INCLIVA, Valencia, Spain, <sup>2</sup>Pediatric Service, Clinical Hospital of Valencia, Valencia, Spain, <sup>3</sup>CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Valencia, Spain.

Abetalipoproteinemia (ABL) is a rare lipid metabolism disease, where mutations in causative known genes can only explain a low percentage of cases. This disease is characterized by very low plasma concentrations of triglycerides and total cholesterol (under 30mg/dl), and undetectable levels of LDL and ApoB. Later in life, ABL is associated with atypical development of retinitis pigmentosa, coagulopathy, column neuropathy and myopathy.

We used whole-exome sequencing (WES) to study a family with two affected children and their asymptomatic parents. Mutations in causative known genes for ABL (MTTP) and for diseases with similar phenotype such as Familial Hypobetalipoproteinemia (APOB, PCSK9 and ANGPTL3) have been previously discarded by Sanger sequencing.

By examining homozygous and compound heterozygous inherited variants, we found that both children carried mutations in TMC6 and SEC23A genes. Although TMC6 does not show a consistent relationship with lipid metabolism, recent studies have demonstrated that SEC23A, an essential component of coat protein complex II (COPII)-coated vesicles, could be involved in the apoB100-lipoproteins exit from the endoplasmic reticulum to the Golgi apparatus.

Besides, SEC23A has been associated with Craniolenticulosternal dysplasia (CLSD), disorder characterized by late-closing fontanelles among other features. Since both children showed unexplained late-closing fontanelles, our study strongly indicate that SEC23A could be a new ABL causative gene not previously described. Functional assays are currently being developed for both genes to confirm these results.

**P06.02****Loss of function of the mitochondrial repressor RIP140 halts axonal degeneration in the peroxisomal disease X-linked adrenoleukodystrophy**

P. Ranea-Robles<sup>1,2</sup>, M. Ruiz<sup>1,2</sup>, J. Galino<sup>1,2</sup>, A. Schlüter<sup>1,2</sup>, I. Ferrer<sup>3</sup>, A. Pujo<sup>1,2,4</sup>, S. Fourcade<sup>1,2</sup>;

<sup>1</sup>IDIBELL, Hospital de Llobregat-Barcelona, Spain, <sup>2</sup>CIBERER U759, Centro de Investigación en Red sobre Enfermedades Raras, Barcelona, Spain, <sup>3</sup>Institute of Neuropathology, Pathologic Anatomy Service, Bellvitge Biomedical Research Institute, IDIBELL, Hospital de Llobregat-Barcelona, Spain, <sup>4</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

X-linked adrenoleukodystrophy (X-ALD) is a rare disease, with fatal prognosis and no satisfactory treatment, characterized by brain inflammatory demyelination and/or axonal degeneration of corticospinal tracts in the spinal cord. It is caused by loss-of-function of the peroxisomal transporter ABCD1. As a result, very long-chain fatty acids (VLCFA) such as C26:0 accumulate in tissues and plasma. The murine model of X-ALD (*Abcd1*) exhibits a late-onset axonal degeneration in spinal cords. We have previously reported that excess of C26:0 produced oxidative stress of mitochondrial origin, which compromises energy metabolism and suppresses the mitochondrial biogenesis pathway driven by the SIRT1/PGC-1α/PPAR network, very early in the physiopathogenetic cascade.

In this study, we have identified RIP140, a novel transcriptional regulator of energy metabolism that antagonizes PGC-1 activation of mitochondria biogenesis, as being overexpressed in the X-ALD mouse spinal cords. We show that RIP140 is directly regulated by C26:0 via an oxidative stress-dependent mechanism. Further, a double knockout mouse X-ALD/RIP140 shows normalized mitochondrial respiration and bioenergetic failure, as well as axonal degeneration and associated locomotor disabilities.

Altogether, these results highlight RIP140 as a novel therapeutic target for X-ALD, and suggest that its pharmacological inhibition may be a valuable strategy to treat this and other axonopathies in which energetic homeostasis and mitochondria biogenesis may be impaired.

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**P06.04****A BCAT2 mutation causes a novel inborn error of metabolism due to mitochondrial branched-chain amino acid aminotransferase deficiency and results in developmental delay and autism**

I. Knerr<sup>1</sup>, J. Urquhart<sup>2</sup>, J. Hughes<sup>1</sup>, Y. Rogers<sup>1</sup>, S. Jones<sup>2</sup>, S. Lynch<sup>1</sup>, E. P. Treacy<sup>1</sup>, P. Mayne<sup>1</sup>, S. Banka<sup>3,2</sup>, S. Olpin<sup>1</sup>;

<sup>1</sup>Temple Street Children's University Hospital, Dublin, Ireland, <sup>2</sup>St Mary's Hospital, Manchester, United Kingdom, <sup>3</sup>University of Manchester, Manchester, United Kingdom,

<sup>4</sup>Sheffield Children's Hospital, Sheffield, United Kingdom.

**Introduction:** Plasma amino acid profile in an adolescent boy with history of early onset developmental delay and autism revealed profoundly elevated levels of branched-chain amino acids (BCAA) - leucine, valine and isoleucine, without an increase in their corresponding branched-chain 2-ketoacids. Notably, alloisoleucine was not detected and Maple Syrup Urine Disease was ruled out. The biochemical phenotype suggested a defect in the first step of BCAA catabolism.

**Results:** Targeted Sanger sequencing of BCAT2 gene that encodes mitochondrial BCAA aminotransferase revealed a novel homozygous inframe deletion (NM\_001190; c136\_147del; p.His46\_Pro49del). Studies conducted on patient's fibroblasts demonstrated a marked reduction in valine oxidation and somewhat impaired leucine oxidation supporting a deficiency of the BCAT enzyme. Protein structural analysis and additional functional studies are ongoing to elucidate the mechanism linking the mutation and enzyme activity.

**Conclusion:** Till date, there is only one reported case of BCAT2 deficiency in the literature that resulted from two compound missense heterozygous BCAT2 mutations in a 25-year-old man with normal development and six-year history of headaches with mild memory impairment (Wang et al 2015). Our findings lead to discovery of a novel inborn error of catabolism of BCAs caused by an inframe BCAT2 deletion resulting in developmental delay and autism.

**References**

1. Wang XL et al. J Inher Metab Dis 2015;38(5):855-61.

**P06.05****Unexpected findings from Next Generation Sequencing (NGS) panel suggesting mitochondrial disorder in two patients.**K. Simenson<sup>1</sup>, S. Pajusalu<sup>1,2</sup>, T. Kahre<sup>1,3</sup>, R. Zordania<sup>1</sup>, R. Rein<sup>2</sup>, K. Öunap<sup>1,2</sup>;<sup>1</sup>Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia,<sup>2</sup>Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, <sup>3</sup>Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia.

Mitochondrial diseases are clinically heterogeneous group of disorders affecting energy production. Due to clinical and genetic complexity it is difficult to recognize them in clinical practice. We present two cases which were clarified by targeted NGS panel.

Case 1: 14-year-old male who was born by Caesarian section due to fetal overgrowth. He has intellectual disability (ID), verbal dyspraxia, mildly decreased muscle tone, bilateral moderate sensorineural hearing loss and seizures. MRI and EEG were normal. We found compound heterozygous mutations in BCS1L gene (c.232A>G, p.Ser78Gly and c.245C>T, p.Ser82Leu; NM\_001257342.1). Mutations in BCS1L gene are associated with mitochondrial respiratory chain complex III deficiency which leads to reduced activity of electron-transport chain and increased production of reactive oxygen species.

Case 2: 15-year-old female, who was born with microcephaly (occipito-frontal circumference 31cm, -3SD). She has dysmorphic features, severe ID, microcephaly, epilepsy and mitral valve prolapse. In infancy lactate and alanine were mildly elevated. CT showed enlargement of lateral ventricle and brain atrophy. Muscle electromicroscopical investigation was normal. We found a novel de novo heterozygous frameshift mutation in PDHA1 gene (c.1014\_1017dup, p.Arg340Leufs\*13, NM\_001173454.1). Mutations in PDHA1 gene are associated with pyruvate dehydrogenase complex deficiency. This causes malfunction of citric acid cycle and deprives the body of energy.

We asked for ID and epilepsy associated genes for case 1, microcephaly and ID associated genes for case 2 from NGS panel. These cases illustrate that NGS is a powerful tool which enables diagnosing patients with non-classical phenotypes and thus sometimes provides a new perspective to clinicians.

**P06.06****Admixture Analysis between allelic effect and HDL-C concentration related to metabolic syndrome (MetS) in Tehran cardio-metabolic genetic study (TCGS)**B. Sedaghatkhayat<sup>1</sup>, M. Fallah<sup>1</sup>, K. Guity<sup>1</sup>, S. Masjoodi<sup>1</sup>, F. Azizi<sup>2</sup>, M. Daneshpour<sup>1</sup>;<sup>1</sup>Cellular and molecular endocrine research center, Research Institute for endocrine sciences Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Endocrine Research Center, Research Institute for endocrine sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Background: HLD-C variation is one of the main phenotypes in most non-communicable disorders like metabolic syndrome. Genetics and environmental factors have undeniable effects on level of HDL-C; among them can be noted to body mass index (BMI) and FTO gene. The current study wants to examine interaction between BMI and FTO and CETP SNPs and their effects on HDL-C level changes in metabolic syndrome.

Material and methods: 1142 subjects were selected from TCGS (%49 male). 382 well defined MetS used as a case and the others used as control. Nine selected polymorphisms from FTO and CETP genes were genotyped by Tetra ARMS-PCR. After adjustment for covariate like age, sex and smoking, the association and interactions between studied alleles and HDL-C and metabolic syndrome in different BMI groups, was tested using plink software and PASW Statistics 18.

Results: Among all nine SNPs, in presence of BMI, lower serum HDL-C had the strongest association with 3 SNPs: rs3764261 (OR: 0.6281, CI95%; 0.6243-0.632, P=2.31\*10-6), rs1800775 (OR: 1.517, CI95%; 1.508-1.526, P=7.15\*10-6) and rs1864163 (OR: 1.062, CI95%; 1.018-1.108, P=5.08\*10-3). Also other SNPs with other parameters of lipid profile showed significance association.

Conclusion: In summary, there are some significance relationships between HDL-C level and studied polymorphisms in the presence of BMI in metabolic syndrome subjects. It seems that the effects of FTO variations on HDL-C level are affected by BMI, and BMI play a important role.

**P06.07****Mutational spectrum of non-PMM2 Congenital Disorders of Glycosylation in the Spanish population**C. Medrano<sup>1</sup>, A. Vega<sup>1</sup>, M. Girós<sup>2</sup>, M. Serrano<sup>3</sup>, M. Ecay<sup>1</sup>, M. Ugarte<sup>1</sup>, B. Pérez<sup>1</sup>, C. Pérez-Cerdá<sup>1</sup>;<sup>1</sup>Centro de Diagnóstico de Enfermedades Moleculares. CBM. Universidad Autónoma de Madrid. CIBERER. IDIPAZ, Madrid, Spain, <sup>2</sup>Servicio BGM, Sección ECM, Hospital Clínico Barcelona, CIBERER, Barcelona, Spain, <sup>3</sup>Dep. Neuropediatría, Hospital San Joan de Déu, CIBERER, Barcelona, Spain.

Congenital disorders of glycosylation (CDG) are a heterogeneous group of disorders that entails defects on proteins of the glycosylation pathways. The majority of them are involved in the N-glycosylation pathway of proteins (N-CDGs), being PMM2-CDG the most frequent disease. Nevertheless, recent studies have revealed that some glycosylation disorders occur with greater frequency than current estimates. The aim of our work was to establish the mutational spectrum of CDG patients in the Spanish population, focusing on non-PMM2-CDG. 83 suspected-CDG patients were selected based on their clinical ground and/or abnormal serum transferrin isoforms. Sanger sequencing and/or enzymatic analysis identified 45 PMM2-CDG patients (54%). The genetic analysis of the remaining 38 suspected non-PMM2-CDG was addressed by Sanger sequencing or targeted exome sequencing of 43 CDG-genes or whole-exome sequencing or TruSightTM One sequencing panel (Illumina). 23 non-PMM2-CDG patients were genetically diagnosed (60.5%), finding pathogenic mutations on 15 different CDG-genes (ALG1, ALG6, ATP6V0A2, B4GALT1, CCDC115, COG7, DOLK, DPAGT1, DPM1, GFPT1, MPI, PGM1, RFT1, SRD5A3, SSR4). All of them are involved in the synthesis of the oligosaccharide linked to dolichol or its transfer onto the protein except ATP6V0A2, B4GALT1, CCDC115 and COG7 which are involved in the processing of the glycan or Golgi homeostasis. Overall, 28 different mutations were identified, 14 of them are novel mutations. In conclusion: next-generation sequencing has improved the diagnosis of these heterogeneous pathologies but reverse phenotyping of the CDG patients will lead an accurate molecular diagnosis because without phenotype information, genome analysis would be of limited medical value. FIS PI11/1254

**P06.08****Development and delivery of an integrated biochemical and genetic testing service for cerebral creatine deficiency syndromes**R. E. Mitchell<sup>1</sup>, I. R. Berry<sup>1</sup>, R. Barski<sup>2</sup>, N. Camm<sup>1</sup>, K. Prescott<sup>1</sup>, C. M. Watson<sup>1</sup>, *Deciphering Developmental Disorders Study*<sup>3</sup>, R. Charlton<sup>1</sup>;<sup>1</sup>Yorkshire Regional Genetics Service, St James's University Hospital, Leeds, United Kingdom, <sup>2</sup>Special Laboratory Medicine, St James's University Hospital, Leeds, United Kingdom, <sup>3</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Cerebral creatine deficiency syndromes (CCDS) are inborn errors of metabolism involving defects in endogenous synthesis and transport of creatine resulting from pathogenic mutations in the genes *GAMT*, *GATM* and *SLC6A8*. CCDS have a broad differential diagnosis; clinical features include intellectual disability, speech delay and seizures. Although rare, CCDS are thought to be under-diagnosed with defects in the creatine transporter, *SLC6A8*, estimated to account for 2% of X-linked intellectual disability and 1% of males with intellectual disability of unknown aetiology. Here, a targeted CCDS sequencing panel was validated for diagnostic use in the Leeds Genetics Laboratory, delivered as an integrated service involving first-line biochemical testing. This service has thus far detected a pathogenic *SLC6A8* mutation in one child with global developmental delay and a homozygous pathogenic *GAMT* mutation in two children with severe speech and language delay, with further familial testing where appropriate. Additionally, Whole Exome Sequencing data were obtained from 1133 trios in the Deciphering Developmental Disorders Study, of which 42% of probands were males with at least one relevant clinical feature and 5% had an X-linked variant reported by the DDD Study. No pathogenic variants in *SLC6A8* were identified in this cohort, consistent with the predicted rarity of CCDS. Nonetheless, this is the first CCDS genetic diagnostic testing offered in a United Kingdom accredited laboratory, uniquely integrated alongside a biochemistry service. It remains likely that CCDS is significantly under-diagnosed and it is therefore anticipated that the service validated here will contribute to an increased diagnostic rate in the UK.

**P06.09****Persistence of positive renal and cardiac effects of migalastat in patients with Fabry disease (FD)-amenable mutations following 30 months of treatment in ATTRACT (Study 012)**

D. G. Bichet<sup>1</sup>, D. P. Germain<sup>2</sup>, R. Giugliani<sup>3</sup>, D. Hughes<sup>4</sup>, R. Schiffmann<sup>5</sup>, W. R. Wilcox<sup>6</sup>, J. P. Castelli<sup>7</sup>, E. S. Cantor<sup>7</sup>, J. Kirk<sup>7</sup>, N. Skuban<sup>7</sup>, J. A. Barth<sup>7</sup>, M. Bechter<sup>8</sup>,

<sup>1</sup>Hôpital du Sacré Coeur, University of Montreal, Montréal, QC, Canada, <sup>2</sup>Hôpital Raymond Poincaré (APHP), University of Versailles-St. Quentin en Yvelines (UVSQ), Garches, France, <sup>3</sup>Medical Genetics Service, Hospital de Clínicas de Porto Alegre, UFRGS, Porto Alegre, Brazil, <sup>4</sup>University College London, London, United Kingdom, <sup>5</sup>Baylor Research Institute, Dallas, TX, United States, <sup>6</sup>Emory University, Atlanta, GA, United States, <sup>7</sup>Amicus Therapeutics, Cranbury, NJ, United States, <sup>8</sup>Amicus Therapeutics UK Limited, Buckinghamshire, United Kingdom.

**Introduction:** FD, an X-linked lysosomal  $\alpha$ -galactosidase A deficiency disorder, leads to substrate accumulation and clinical sequelae. Migalastat, an oral pharmacological chaperone, stabilizes amenable mutant forms of  $\alpha$ -galactosidase A in FD patients, increasing lysosomal activity. ATTRACT is an 18-month, open-label study, comprising a randomized comparison study of ERT-experienced patients switching to 150 mg QOD migalastat HCl (n=36) or remaining on ERT (n=21) and a 12-month open-label extension (OLE) with migalastat.

**Materials and Methods:** In the migalastat group, 31 patients with amenable mutations completed the 18-month period and entered 12-month OLE; 49 with amenable mutations received  $\geq 1$  migalastat dose during the combined 30 months.

**Results:** In patients receiving migalastat for 30 months, the mean (95% CI) annualized rate of change (mL/min/1.73m<sup>2</sup>) from baseline (cbl) in eGFR/CKD-Epi and mGFR/ihexol were  $-1.7$  ( $-2.7$ ,  $-0.8$ ) and  $-2.7$  ( $-4.8$ ,  $-0.6$ ); rates were comparable to those reported in patients receiving ERT for 18 months. In patients receiving migalastat for 30 months, the mean (95% CI) cbl in LVMi (g/m<sup>2</sup>) were  $-3.8$  ( $-8.9$ ,  $1.3$ ) for all patients and  $-10.0$  ( $-16.6$ ,  $-3.3$ ) for those with LVH. For ERT-treated patients, previously reported 18-month changes in LVMi were  $-2.0$  ( $-11.0$ ,  $7.0$ ) for all patients and  $+4.5$  ( $-20.9$ ,  $29.9$ ) for patients with LVH. LVMi reduction from baseline to month 30 in patients with baseline LVH treated with migalastat was statistically significant.

**Conclusion:** In patients switched from ERT, the renal and cardiac effects of migalastat persist over 30 months, making migalastat a promising first-in-class oral chaperone treatment for male/female patients with amenable mutations.

**P06.10****Biochemical peculiarities and clinical aspects in three Romanian patients with classic galactosemia**

A. A. Chis<sup>1</sup>, A. Nicolescu<sup>2</sup>, C. Deleanu<sup>3</sup>, S. Tatar<sup>4</sup>, R. Pop<sup>4</sup>, R. Vulturaru<sup>4</sup>,

<sup>1</sup>Univ. of Medicine and Pharmacy, Cluj-Napoca, Romania, <sup>2</sup>Ponț Institute of Macromolecular Chemistry of the Romanian Academy, Iasi, Romania, "C.D. Nenitescu" Institute of Organic Chemistry of the Romanian Academy, Bucharest, Romania, Iasi, Romania, <sup>3</sup>P. Ponț Institute of Macromolecular Chemistry of the Romanian Academy, Iasi, Romania, "C.D. Nenitescu" Institute of Organic Chemistry of the Romanian Academy, Bucharest, Romania, Iasi, Romania, <sup>4</sup>IIIrd Pediatric Clinic, Cluj-Napoca, Romania.

Classic galactosemia is a hereditary disorder of galactose metabolism caused by a deficiency in GALT (galactose-1-phosphate uridyl transferase). Patients diagnosed with this disorder show a potentially fatal phenotype after galactose intake in the first days of life. A galactose-restricted diet quickly resolves this acute symptoms. The exact mechanism(s) causing the complications (cognitive impairment and in females - ovarian insufficiency) and the time window in which the damage occur remain uncertain.

The national newborn screening in our country is limited to PKU and Congenital hypothyroidism, and this is why a fast analysis for urinary biomarkers in patients suspected for genetic metabolic disease is an emergency. We present the biochemical abnormalities, the clinical pictures and evolution in three newborn patients diagnosed with classic galactosemia after the first days of life; concentration of urinary galactose (between 9.890 and 11.897 mmol/molCreatinine) and galactitol (between 4.380 and 9.832 mmol/molCreatinine) were measured using an Bruker-Avance-400 MHz Spectrometer; the 1H-NMR spectrum of urine helped in a fast diagnosis but also in comparing the biochemical peculiarities with clinical pictures in our patients.

Metabolic profiling is an essential component that along with genetics, transcriptomics and proteomics data will permit a detailed description of the interactions between metabolites, proteins, transcripts and genes in the disease continuum.

Our cases will contribute to a better understanding of biochemical phenotype and clinical effects. This report highlight the importance of an extensi-

on of New Born Screening (NBS) in Romania and, as well, the importance of early confirmation of the diagnosis for family counseling.

**P06.11****COQ2-associated primary coenzyme Q10 deficiency: functional characterization of the human gene and establishment of genotype-phenotype correlations**

E. Trevisson<sup>1</sup>, M. A. Desbats<sup>1</sup>, V. Morbidoni<sup>1</sup>, M. Silic-Benussi<sup>2</sup>, V. Ciminale<sup>2</sup>, F. Pierrel<sup>3</sup>, P. Navas<sup>4</sup>, L. Salviati<sup>1</sup>,

<sup>1</sup>Clinical Genetics, Department of Women's and Children's Health, University of Padova, Padova, Italy, <sup>2</sup>Istituto Oncologico Veneto-IRCCS (IOV), Padova, Italy, Padova, Italy,

<sup>3</sup>Commissariat à l'Energie Atomique (CEA), Institut de Recherches en Technologies et

Sciences pour le Vivant, Laboratoire Chimie et Biologie des Métaux, Grenoble, France,

<sup>4</sup>Centro Andaluz de Biología del Desarrollo, University Pablo de Olavide, Sevilla, Spain.

**Introduction:** COQ2 encodes para-hydroxybenzoate polyprenyltransferase, that catalyzes the second step of the final reaction sequence of Coenzyme Q10 (CoQ) biosynthesis. A homozygous mutation in COQ2 was the first molecular defect reported in a family with primary CoQ deficiency in 2006, followed by the identification of other 10 families. Their phenotypes vary widely but genotype-phenotype correlations remain to be clarified.

The structure of human COQ2 has been inferred by sequentially cloning the 5' region (amplified from genomic DNA) and the 3' region obtained from cDNA and the reported human sequence shows 4 in frame ATG initiation codons.

**Methods:** we employed *in silico* and experimental analyses to characterize the 5' region of COQ2, to analyze the topology and subcellular localization of Coq2p and set up a yeast model to validate COQ2 mutations.

**Results:** We provided experimental evidence showing that the functional human COQ2 transcript is shorter than what was previously thought and that Coq2 is a mitochondrial protein with the C terminus facing the inter-membrane space. We engineered all the known COQ2 missense alleles into the *S.cerevisiae* orthologue to analyze the respiratory phenotype in the deleted strain. Our model proved to be simple and reproducible and demonstrated the hypomorphic nature of all mutations. We also showed that the residual activity (measured by CoQ content in yeast) clearly correlates with patients' clinical phenotype.

**Conclusions:** Our data support the hypothesis that the affected tissues display a specific sensitivity to different levels of CoQ10 deficiency.

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**P06.12****Unusual phenotype with a novel homozygous SLC25A1 mutation**

O. Wormser<sup>1</sup>, I. Cohen<sup>1</sup>, Y. Yogeve<sup>1</sup>, O. Staretz-Chacham<sup>2</sup>, O. S. Birk<sup>3,1</sup>,

<sup>1</sup>Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev (NIBN), Ben Gurion University, Beer-Sheva, Israel, <sup>2</sup>Pediatric Metabolic Unit, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel, Beer-Sheva, Israel,

<sup>3</sup>Genetics Institute, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel, Beer-Sheva, Israel.

Five infants of two related consanguineous Bedouin families presented with a syndrome of neonatal-onset encephalopathy with severe muscular weakness, intractable seizures, respiratory distress and delayed psychomotor development, culminating in early death. Muscle biopsy showed evidence of complex V mitochondrial disease, and brain imaging demonstrated ventriculomegaly with thinning of corpus callosum. Homozygosity at loci of genes known to be associated with mitochondrial complex V deficiency was ruled out using polymorphic markers. Whole exome sequencing data of an affected individual were filtered for known benign variants using open access databases (ClinVar, HapMap, EVS and 1000 genomes) and our in-house data of over 100 ethnically matched exomes. Segregation analysis ruled out all the putatively deleterious homozygous mutations found, except for a single homozygous variant: NM\_005984: c.713A>G in SLC25A1, resulting in a p.N238S missense mutation. The mutation fully segregated in the family as expected in recessive heredity. Mutations in SLC25A1 have been shown to be associated with a rare neuro-metabolic disorder of combined D,L-2-hydroxyglutaric aciduria (DL-2HGA; OMIM #615182). To the best of our knowledge this is the first study showing a mutation in SLC25A1 causing documented mitochondrial complex V deficiency.

## P06.13

### Congenital disorder of glycosylation type Iq: Report of a patient with an SRD5A3 mutation identified on whole exome sequencing and review of the literature

A. Hanson-Kahn<sup>1</sup>, K. Liker<sup>1</sup>, L. Slattery<sup>2</sup>, M. Manning<sup>1</sup>;

<sup>1</sup>Stanford University, Stanford, CA, United States, <sup>2</sup>Lucile Packard Children's Hospital, Palo Alto, CA, United States.

Congenital disorders of glycosylation are multisystem disorders caused by abnormal glycosylation of proteins. Many subtypes have been described. Patients with type Iq, caused by mutations in the SRD5A3 gene, have ophthalmologic abnormalities, ataxia, intellectual disability, speech delay and hypotonia. We report the first patient with type Iq who was diagnosed by clinical whole exome sequencing.

The patient was referred for evaluation of nystagmus noted at 3 months, optic nerve atrophy, hypotonia and global developmental delay. Pregnancy and delivery were uncomplicated. The patient rolled over at 8 months, sat at 10 months and walked at 24 months. She has no words at 2 years. ERG, brain MRI and EEG are normal. She is an only child, and her parents are first cousins.

Whole exome sequencing identified a c.57G>A (p.W19X) variant in SRD5A3, which has thusfar only been described in 5 patients. Our patient's features are similar to the other individuals described, although she does not have ataxia. Transferrin isoelectric focusing was consistent with a type I profile. Ophthalmologic findings in patients with c.57G>A seem to be restricted to optic nerve atrophy, nystagmus and strabismus. Ataxia is less common, and cerebellar malformation may be absent. Intellectual disability is described as being mild.

We review the literature about the Iq subtype, further delineate the clinical phenotype of the c.57A>G mutation, and propose that testing for congenital disorders of glycosylation type 1q be considered in any patient with nystagmus, strabismus or optic nerve atrophy, hypotonia and mild intellectual disability.

## P06.14

### Gene defects underlying the congenital lactic acidosis

I. Bravo-Alonso, R. Navarrete, A. Vega, B. Merinero, C. Pérez-Cerdá, M. Ugarte, B. Pérez, P. Rodríguez-Pombo;

Centro de Diagnóstico de Enfermedades Moleculares (CEDEM), Centro de Biología molecular Severo Ochoa (CSIC-UAM), CIBERER-ISCIII, IDIPAZ, Universidad Autónoma de Madrid, Madrid, Spain.

Congenital Lactic Acidosis (CLA) is the biochemical hallmark of a heterogeneous number of non-genetic and genetic conditions. A large number of defects related to the disruption of normal pyruvate metabolism and/or mitochondrial function, with a growing list of mitochondrial diseases caused by different nuclear gene defects, makes the molecular diagnosis a labor-intensive work that requires a systematic multidisciplinary approach. Herein, we present data relative to the massive-parallel sequencing, using the Illumina® clinical-exome TruSight™ One Sequencing panel, of a cohort of 29 patients, all selected for having neonatal/infantile encephalopathy with CLA. The clinically significant deemed variants found were confirmed by subsequent Sanger sequencing. In thirteen out of the 29 patients, we yield the molecular diagnosis, with mutations in PDHA1 (3), PDHX (1), PHKA2 (1), ACAD9 (1), TK2 (1), NDUFS4 (1), FOXRED1 (1), GFM1 (1), TSFM (1), PDSS1 (1) and COQ2 (1) genes. In silico predictions supported a pathogenic role in each case. Segregation analysis in parents' DNA confirmed their carrier condition. The overall rate of positive diagnosis was 45%. In absence of previous diagnostic work-up, an impaired real-time oxygen consumption rate in cultured intact patients' fibroblasts is been used as standard method to evaluate the mitochondrial functionality when samples were available. Underscoring the genetic heterogeneity of congenital lactic acidosis, our findings implicate 11 different genes in 29 patients. We conclude that targeted exome sequencing enhances the ability to identify the underlying gene mutation when a well-sustained clinical suspicion has been previously established.

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## P06.15

### Lack of association between increased mitochondrial DNA4977 deletion and ATP levels of sputum cells from chronic obstructive pulmonary disease patients vs healthy smokers

Y. Müşteri Oltulu<sup>1</sup>, A. Karimova<sup>2</sup>, D. Ustek<sup>3</sup>, M. Kara<sup>4</sup>, B. Tutluoglu<sup>5</sup>, H. Azaklı<sup>3</sup>, I. Onaran<sup>2</sup>;

<sup>1</sup>Health Sciences Faculty, Department of Nutrition and Dietetics, Biruni University, Istanbul, Turkey, Istanbul, Turkey, <sup>2</sup>Department of Medical Biology and Genetics, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey, Istanbul, Turkey, <sup>3</sup>Istanbul Medipol University, Department of Medical Genetics, School of

Medicine, Istanbul, Turkey, Istanbul, Turkey, <sup>4</sup>Department of Genetics, School of Medicine, Mugla University, Mugla, Turkey, Mugla, Turkey, <sup>5</sup>Department of Pneumology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey, Istanbul, Turkey.

**Introduction:** In this study we looked at smokers with and without chronic obstructive pulmonary disease (COPD) patients in order to evaluate the incidence of 4977 bp mtDNA (mtDNA4977) deletion and mtDNA copy number in sputum cells and in peripheral blood leukocytes (PBLs) in relation to mitochondrial function and oxidative stress status.

**Materials and Methods:** Twenty-five COPD patients who were current smokers, 22 smokers and 23 healthy nonsmokers (for only PBLs studies) participated in this study. The 4977-bp deletion was detected using a quantitative real time PCR.

**Results:** The frequency of the mtDNA4977 was significantly higher in the sputum cells of patients with COPD compared to smokers without COPD ( $p<0.0001$ ). This difference was not observed in PBLs. Levels of cellular oxidative stress were significantly higher in the sputum cells of subjects with COPD than in the smoker group. However, mtDNA copy number, mitochondrial membrane potential ( $\Delta\Psi_m$ ) and cellular ATP levels in PBLs and sputum cells were not significantly different between the studied groups. The Pearson analysis revealed no correlations between the accumulation of mtDNA4977, and intracellular ATP content and  $\Delta\Psi_m$  values of the sputum cells, although there was a positive correlation between increased mtDNA4977 and the levels of cellular oxidative stress in COPD patients ( $r:0.80$ ,  $p<0.0001$ ).

**Conclusions:** Our studies may suggest that the accumulation of mtDNA4977 in the sputum cells of smokers with COPD does not seem to have an important impact on mitochondrial dysfunction in relation to ATP production and  $\Delta\Psi_m$  when compared to those of healthy smokers.

## P06.16

### Treatment of the CTNS W138X nonsense mutation with nonsense suppressor drugs

E. J. Brasell<sup>1</sup>, L. Chu<sup>2</sup>, D. Iglesias<sup>3</sup>, Y. Yamanaka<sup>4</sup>, P. Goodyer<sup>2</sup>;

<sup>1</sup>McGill University, Montreal, QC, Canada, <sup>2</sup>McGill University Health Centre, Montreal, QC, Canada, <sup>3</sup>Genome Quebec, Montreal, QC, Canada.

**Introduction:** Cystinosis is caused by biallelic mutations of the cystinosin (CTNS) gene, encoding a lysosomal cystine transporter. Intralysosomal cystine accumulation drives progressive organ dysfunction. In Quebec, about 50% of cystinosis patients harbor the W138X nonsense mutation causing a stop codon in exon 7. Nonsense mutations trigger nonsense-mediated decay (NMD) of mutant transcripts and inhibit protein translation.

**Materials and Methods:** Aminoglycosides, such as gentamicin (G418), have nonsense suppressor activity, which permits translational read-through of premature stop codons. To test the effect of G418 on the W138X mutation, we treated *CTNS<sup>W138X/W138X</sup>* human fibroblasts for 24h in culture and examined CTNS expression and intracellular cystine levels. Using ZFN technology, we also generated a *Ctns* nonsense mutant mouse (Y226X) for in vivo studies of aminoglycoside-like compounds.

**Results:** After 24h treatment with G418, *CTNS* mRNA transcript levels in *CTNS<sup>W138X/W138X</sup>* fibroblasts increased to normal levels. Furthermore, G418 reduced intracellular cystine levels by 60%. These results suggest that G418 reduces NMD of *CTNS<sup>W138X/W138X</sup>* transcript and allows production of functional CTNS. In addition, we confirmed that *Ctns<sup>Y226X/Y226X</sup>* mice have decreased levels of *Ctns* transcript and intracellular cystine accumulation, as well as glucosuria and early swan-neck lesions of the proximal tubule by 6 months of age.

**Conclusions:** G418 promotes read-through of the *CTNS* W138X mutation, producing sufficient CTNS protein for cystine efflux from lysosomes. This suggests that nonsense suppressor compounds may offer an effective therapeutic strategy for cystinosis involving nonsense mutations. Our *Ctns<sup>Y226X/Y226X</sup>* mice provide a pre-clinical model for screening next-generation nonsense suppressor drugs.

**Fellowship:** Cystinosis Research Foundation.

## P06.17

### Association of ENPP1 polymorphism K121Q with type 2 diabetes mellitus and stroke in Ukrainian population

O. Obukhova, V. Harbuzova, I. Marchenko;

Sumy State University, Sumy, Ukraine.

**Introduction:** T2DM is a complex polygenic disorder in which common genetic variants interact with environmental factors to unmask the disease. ENPP1 is an II transmembrane glycoprotein that inhibits insulin signaling by direct interaction with the insulin receptor beta subunit. This inhibition is enhanced by the minor Q allele of the K121Q polymorphism (rs1044498)

in the gene *ENPP1*, and causes insulin resistance and diabetes. A stroke is a medical condition that can affect diabetes. Patients with diabetes who suffer a stroke are at greater risk of both disability and death.

**Materials and Methods:** Venous blood of 163 patients with T2DM and 110 healthy individuals (control group) was used for genotyping. Analysis of *ENPP1* polymorphism K121Q (rs1044498) was examined by PCR-RFLP with the following restriction fragment length analysis of the allocation of them by electrophoresis in agarose gel. Statistical analysis was performed by using the software package SPSS-17. The value of  $P < 0.05$  was considered as significant.

**Results:** Using the Pearson criterion was reveal association between the K121Q polymorphism of *ENPP1* gene and the development of T2DM in stroke and non-stroke patients. It was shown that in patients with stroke value homozygotes for the major allele (K/K) and minor allele carriers (K/Q+Q/Q) is 48.8% and 51.2%, while in the non-stroke patients - 70.5 % and 29.5% respectively ( $P = 0.012$ ).

**Conclusions:** In diabetic patients with genotype K/Q+Q/Q risk of stroke was significantly higher than in those with K/K genotype in Ukrainian population.

#### P06.18

#### Variants Across Multiple Candidate Genes Confer Susceptibility to Diabetic Retinopathy in a North Indian Population

*R. Sikka<sup>1</sup>, J. Chhablani<sup>2</sup>, A. J. S. Bhanwer<sup>3</sup>, S. Chakrabarti<sup>4</sup>,*

<sup>1</sup>Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India,

<sup>2</sup>Smt Kanuri Santhamma Centre for Vitreo Retinal Diseases, Hyderabad, Telangana, India,

<sup>3</sup>Guru Nanak Dev University, Amritsar, Punjab, India, <sup>4</sup>LV Prasad Eye Research Institute, Hyderabad, Telangana, India.

Over the last two decades, there have been tremendous efforts in characterizing the molecular mechanisms underlying complex disorders like diabetes along with its associated variant, diabetic retinopathy (DR). These approaches were largely based on Genome Wide Association Studies and candidate gene screenings for identifying the associated biomarkers. These studies were limited largely due to population substructuring along with the vagaries of small sample size. In the present study, we performed targeted genome screening of 97 SNPs across in 48 candidate genes involved in the metabolic pathways leading to DR. We analyzed these variants in a cohort of 848 subjects (414 DR cases along with gender and ethnicity matched 434 controls above the age of 50 years) using Sequenome MassARRAY technology. Strong genetic associations were observed in *TCF7L2*; three intergenic variants; *ADIPOQ* and *TLR-4* ( $p < 0.05$ ) with DR. Further, the clinical and anthropometric data revealed that the history of hypertension [ $p < 0.001$ ; OR=13.64 (9.15=20.34)], male gender [ $p=0.003$ ; OR=1.86 (1.22-2.81)] and BMI [ $p < 0.001$ ; OR=1.31 (1.17-1.46)] were the main risk factors for DR. These results provide strong evidence of association of variants in these genes with susceptibility to DR.

#### P06.19

#### Increased DNA methylation caused by obesity as a key factor for insulin resistance development

*M. Malodobra-Mazur, D. Bednarska-Chabowska, R. Adamiec, T. Dobosz,*

*Wroclaw Medical University, Wroclaw, Poland.*

**Introduction:** Insulin resistance and type 2 diabetes are leading problems of modern civilization. In pathogenesis of both disorders key role is played by obesity. Numerous data implicate obesity with DNA hypermethylation including genes regulating insulin sensitivity, however there are only a few reports concerning promoter's methylation of insulin pathway genes. The aim of our work was to assess the rate of global and site-specific DNA methylation of main insulin pathway genes in obese insulin resistant patients. **Methods:** Global and site-specific DNA methylation was analyzed in lymphocytes and adipose tissue of insulin resistant subjects and controls using EpiJet DNA Methylation Analysis Kit. Site-specific DNA methylation was analyzed within promoters of *INSR*, *PIK3R1* and *SLC2A4* using Real-Time PCR. **Results:** Strong positive correlation between global DNA methylation and BMI was displayed in both types of investigated tissues. Simultaneously, DNA methylation positively correlated with glucose level and insulin resistance ratios (HOMA-IR, QUICKI) in both tissues. Furthermore, increased promoter methylation of analyzed genes was noticed in visceral adipose tissue of insulin resistant patients comparing to controls. What's more, the promoter methylation rate of *INSR* and *SLC2A4* positively correlated with glucose level and insulin resistance and negatively with gene expression. Promoters' methylation status of all genes correlated positively with BMI. **Conclusions:** Obtained results indicate the role of DNA hypermethylation in insulin resistance. Aberrant profile of promoters methylation was shown

for numerous genes regulating insulin sensitivity including *ADIPOQ*, *LPL* or *PPARy*. Present study showed the association between promoters methylation of main insulin pathway genes and insulin resistance.

#### P06.21

#### Elucidation of the molecular process underlying mutant alpha-galactosidase A degradation in Fabry disease

*A. Pockrandt<sup>1</sup>, C. Zheng<sup>1,2</sup>, C. Bohl<sup>1</sup>, J. Lukas<sup>1</sup>, A. Rolfs<sup>1,3</sup>,*

<sup>1</sup>Albrecht-Kossel-Institute for Neuroregeneration, Rostock, Germany, <sup>2</sup>Leibniz Institute for Catalysis, Rostock, Germany, <sup>3</sup>Centogene AG, The Rare Disease Company, Rostock, Germany.

Lysosomal storage disorders (LSD) are a group of variant inherited metabolic diseases caused by protein deficiencies. Lysosomal proteins undergo intracellular processing and trafficking. Mutant proteins in Gaucher and Niemann-Pick Typ C1 disease are exposed to the ER-associated degradation (ERAD). In the present study we intend to investigate on the molecular process underlying inadvertent pre-mature degradation of mutant  $\alpha$ -galactosidase A enzyme ( $\alpha$ -gal A, OMIM \* 300644) in Fabry disease (OMIM # 301500).

We used MALDI TOF mass spectrometry to identify ubiquitinated lysine moieties 130, 240 and 426 of  $\alpha$ -gal A that would lead to proteasomal targeting. Substitution of these three lysines reduced ubiquitination of the enzyme. Furthermore, we over-expressed mutant  $\alpha$ -gal A in HEK293H cells and applied inhibitors of cellular ubiquitination and the proteasome, summarised as Ubiquitin/Proteasome System inhibitors (UPSi) in order to prevent the degradation process. Usage of two UPSi, MG132 and Rosiglitazone, led to an twofold increased enzyme level suggesting a potential clinical relevance for this compound class. In search of intracellular binding partners via immunoprecipitation the direct physical interaction of  $\alpha$ -gal A to different ER resident chaperones (e.g. Calnexin and BiP/GRP78) was confirmed. Further studies will be performed to identify E3 ubiquitin ligase outfit involved in mutant  $\alpha$ -gal A ubiquitination and degradation in disease state.

#### P06.23

#### Use of next generation sequencing for the diagnosis of familial hypercholesterolemia (FH)

*S. Martínez-Figueroa, R. Roig, L. Garrido, A. Beteta, À. García-Osuna, F. Blanco-Vaca;*  
*Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.*

**Introduction:** More than 1500 mutations causing FH are known. Their detection is classically performed by Sanger sequencing and MLPA of the LDLR. Between 2008 and 2012, we used a microarray (LIPOchip®) for detecting the (around 250) most frequent mutations in the Spanish population. Because of the high potential mutation heterogeneity, during 2013 we tested the Next Generation Sequencing (NGS) Roche 454 based SEQPRO LIPO RS®. **Material and Methods:** The SEQPRO LIPO RS kit amplifies 48 amplicons covering the coding regions of the LDLR, PCSK9 and LDLRAP1 and 2 regions of APOB. The SEQPRO LIPO RS software automatically generates variants lists that were compared to a Mutation Database to provide pathogenic significance. 160 samples of FH patients from Catalonia were screened with this kit.

**Results:** DNA was obtained from patients diagnosed used the Dutch MedPed criteria. 27 samples were diagnosed as possible FH (values between 3 and 5), 55 as probable FH (values of 6 and 7), 55 as definite FH (values  $\geq 8$ ). No values were indicated for 23 patients. Mutations were found in 66 samples, representing 41% of all samples. Forty-five different mutations were found, 32 of them being unique, all in the LDLR gene except one mutation in APOB (p.R3527Q). Most mutations were amino acid changes or lead to null alleles. Carriers of mutations whose pathogenicity was already demonstrated showed a higher Dutch Medped than carriers of variants of unknown significance (9.34 vs 6.44).

**Conclusion:** This NGS-based kit was proven adequate for FH mutation detection.

**P06.24****High dietary folate during pregnancy leads to pseudo-MTHFR deficiency, changes in choline metabolites and short-term memory impairment in offspring**

*R. H. Bahous<sup>1</sup>, N. M. Jadavji<sup>2</sup>, M. Cosín-Tomás<sup>3</sup>, L. Deng<sup>4</sup>, J. Lu<sup>5</sup>, O. Malysheva<sup>6</sup>, M. Pallàs<sup>3</sup>, P. Kaliman<sup>7,8</sup>, M. Caudill<sup>9</sup>, R. Rozen<sup>1,4</sup>;*

<sup>1</sup>Department of Human Genetics, McGill University, Montreal, QC, Canada, <sup>2</sup>Department of Neuroscience, Carleton University, Ottawa, ON, Canada, <sup>3</sup>Pharmacology Unit, Faculty of Pharmacy, Institut de Neurociència Universitat de Barcelona (IBUB), Nucli Universitari de Pedralbes, Barcelona, Spain, <sup>4</sup>Department of Pediatrics, McGill University Health Center, Montreal, QC, Canada, <sup>5</sup>Department of Medicine, McGill University, Montreal, QC, Canada, <sup>6</sup>Division of Nutritional Sciences and Genomics, Cornell University, Ithaca, NY, United States, <sup>7</sup>Institute of Biomedical Investigation of Barcelona, Spanish National Research Council, Barcelona, Spain, <sup>8</sup>Center for Mind and Brain, University of California Davis, Davis, CA, United States.

**Introduction:** Severe deficiency of methylenetetrahydrofolate reductase (MTHFR) in mice is associated with short-term memory impairment and disturbed choline metabolism. Folate intake has increased in the general population, particularly in women of childbearing age, due to food fortification and use of high dosage vitamin supplements. The goal of this study was to determine whether high dietary folate during pregnancy and lactation would affect brain function in offspring.

**Materials and Methods:** Female mice were placed on control diets or folic acid-supplemented diets prior to mating, which were maintained during pregnancy and lactation. Embryos were collected at E17.5 or pregnancy was carried to term. Male offspring were evaluated for memory impairment at 3 weeks of age using the novel object recognition test.

**Results:** Pups born to mothers on supplemented diet showed visual short-term memory impairment. In the livers of mothers and pups, MTHFR protein levels were significantly decreased and the ratio of the phosphorylated (less active): non-phosphorylated isoform was increased. Phosphocholine was decreased in livers of pups and glycerophosphocholine was decreased in offspring hippocampus. In the embryos of females on the supplemented diet, we observed growth delay, decreased MTHFR protein in livers and a decrease in some choline metabolites (choline, betaine and phosphocholine).

**Conclusions:** We suggest that high folate intake during pregnancy leads to pseudo-MTHFR deficiency, disturbs choline metabolism and results in memory impairment in offspring. These findings contribute to increased awareness of the unintended negative consequences of folic acid oversupplementation.

**Grants:** Fellowship (Faculty of Medicine-McGill) to RB and CIHR grant to RR.

**P06.25****Evaluation of neopterin as a potential biomarker of Gaucher disease**

*C. Drugan, T. Drugan, N. Miron, C. Cătană;*

*Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania.*

**Introduction:** Gaucher disease, one of the most prevalent lysosomal storage disorders, is caused by a recessively inherited deficiency of the lysosomal enzyme glucuronidase, encoded by the *GBA1* gene. We investigated the usefulness of neopterin, as a potential biomarker in Gaucher disease and analysed its evolution in response to enzyme replacement therapy.

**Materials and methods:** We measured plasma neopterin levels in 31 patients with non-neuronopathic Gaucher disease, before and after the onset of enzyme replacement therapy and compared the results with those of 18 healthy controls. Plasma chitotriosidase activity served as a reference biomarker, against which we evaluated the evolution of neopterin.

**Results:** In treatment-naïve patients, neopterin levels were significantly increased (mean  $11.90 \pm 5.82$  nM), compared with controls ( $6.63 \pm 5.59$  nM), and returned to normal levels ( $6.92 \pm 4.66$  nM) after average interval of 3.8 years of macrophage-targeted recombinant enzyme substitution. Receiver operating characteristic analysis of the diagnostic value of neopterin indicated a cut-off value of 7.613 nM, corresponding to an area under the curve of 0.780 and indicating a good discrimination capacity, with a sensitivity of 0.774 and a specificity of 0.778.

**Conclusions:** Plasma neopterin levels reflect the global accumulation of Gaucher cells and the extent of chronic immune activation in this disorder. Circulating neopterin may be considered an alternative storage cell biomarker in Gaucher disease, especially in chitotriosidase-deficient patients.

**P06.26****Glycogen Storage Disease type IXa: a new variant in PHKA2 detected by Next Generation Sequencing**

*C. Rodríguez Jiménez<sup>1</sup>, F. Santos<sup>1</sup>, Á. Campos<sup>1</sup>, C. Camarena<sup>2</sup>, D. LLedín<sup>2</sup>, E. Sevilla<sup>1</sup>, A. Carazo<sup>1</sup>, E. Vallespín<sup>1</sup>, Á. Del Pozo<sup>1</sup>, R. Mena<sup>1</sup>, P. Lapunzina<sup>1</sup>, S. Rodríguez Núñez<sup>1</sup>;*

<sup>1</sup>INGEMM, Madrid, Spain, <sup>2</sup>Hospital La Paz, Madrid, Spain.

**INTRODUCTION:** Glycogen storage disease (GSD) type IX (MIM: 30600) results from a deficiency in hepatic phosphorylase kinase (PHK) activity, accounting for  $\sim 1/100000$  births. PHK is a decahexameric enzyme composed of four subunits,  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  encoded by four different genes: PHKA2, PHKB, PHKG and CALM, respectively. Mutations in PHKA2 (MIM 300798) cause the most common subtype, X-linked recessive GSD IXa (75% of all GSD IX). Herein, we report a patient with clinical symptoms compatible with GSD VI or IX.

**PATIENT AND METHODS:** A four-year old patient with hepatomegaly, growth retardation, elevation of liver enzymes, hyperlipidaemia and mild fasting hypoglycaemia with hyperketosis was remitted to our service. PYGL, PHKA1, PHKA2, PHKB and PHKG2 were analyzed by Next Generation Sequencing (NGS).

**RESULTS:** We identified the previously undescribed hemizygous variant PHKA2:exon18: c.1963G>A. In silico analyses showed two possible consequences: 1st -missense variant, p.Glu655Lys, affecting a highly conserved amino acid and predicted to be likely pathogenic; 2nd - Splicing variant: MaxEnt, NNSPLICE, GeneSplicer, SliceSiteFinder-like predict that this variant disrupt the ex18 canonical splice donor site, probably resulting in an aberrant protein. The variant was absent in the mother, thus, it is likely to have arisen as "de novo" event.

**CONCLUSIONS:** NGS helped us to improve GSD clinical diagnosis, allowing the simultaneous analysis of several genes. The novel hemizygous variant PHKA2:c.1963G>A, could explain the GSD IXa phenotype observed in our patient. Functional studies should be performed to determine and confirm the pathogenic mechanism, in order to provide genetic counselling to the family.

**P06.27****Identification of two new variants at the codon 281 of HFE-1 gene**

*V. Geromel, N. Couprie, P. Mouty, D. Martinez, L. Raymond, G. Egea;*  
*BIOMNIS, Lyon, France.*

In France, HFE hereditary hemochromatosis counts for 95 % of hereditary iron overload. The homozygosity for the p.Cys282Tyr mutation in the HFE gene leads to hepcidin deficiency, enhanced body iron storage and clinical impact. In Biomnis, the detection of the p.Cys282Tyr allele is based on real-time PCR using Taqman® probes.

Two compound heterozygous states for the p.Cys282Tyr mutant and either the p.His63Asp or the p.Ser65Cys variant alleles are commonly screened although they are associated with mild iron level increase. In recent years several new mutations in HFE gene have been described: for instance a novel C282Y/R226G compound heterozygous state has been found displaying the biochemical hemochromatosis phenotype.

In the course of our genotyping technique for p.Cys282Tyr allele, we have detected abnormal profiles for two patients. A Sanger sequencing has been performed and two new variants have been identified at the 281 codon. For patient 1, the p.Thr281Lys has been found associated with the p.Cys282Tyr and p.His63Asp at the heterozygous states, and for patient 2, the variant p.Thr281Met turned out to be linked to the p.His63Asp allele at the heterozygous state.

Both new mutations do not affect the normal Cys225S-S282Cys disulfide bridge in the HFE protein. However while the p.Thr281Lys allele in patient 1 could show the same specificities as a neutral and hypomorphic variant, the p.Thr281Met allele, related to a marked hemochromatosis phenotype in patient 2, could be consistent with a deleterious character. Further investigations of that mutant protein will be required.

**P06.28****Phenylalanine hydroxylase deficiency in southern Italy: genotype-phenotype correlations, BH4 responsiveness and identification of a novel mutant PAH allele**

*R. Trunzo, R. Santacroce, A. Leccese, G. De Girolamo, M. Margaglione;*  
*Laboratory of Human Genetics, Foggia, Italy.*

Hyperphenylalaninemias (HPAs), the most common inborn error of metabolism (1:10,000), are recessively inherited and caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH converts L-Phenylalanine into L-Tyrosine. PAH block results in accumulation of L-Phe and

toxic metabolites. The mutational spectrum of PAH gene in Southern Italy have been documented elsewhere, but in particular from Campania and Sicily. We sequenced the PAH gene and genotype-phenotype correlations and genotype-based prediction of BH4 responsiveness in 37 PKU patients from Puglia were done. The mutational spectrum included 30 variants with c.1066-11G>A being the most frequent (12,16%). One new variant, c.870 T>G was found. The genotype-phenotype correlation was concordant in 90%. The genotype-based prediction to BH4-responsiveness was around 40% in our patients; thus, this information could be useful for the selection of candidates for BH4 therapy. The present study enlarges the molecular epidemiology of PAH mutations, particularly with respect to Southern Italy. Our data reinforce the wide heterogeneity of PAH mutations in PKU patients and moreover, reveal a good correlation, with patients carrying null mutations in both alleles showing the highest degree of concordance with the most severe phenotypes as previously reported.

Finally, the increasing information obtained in this and other studies will improve the prediction of the evolution of the disease and the diagnostic applicability of mutational analysis. The genotyping of patients became important, not only because of the definitive diagnosis and prediction of the optimal diet, but also to point out those patients that could benefit from new therapeutic approach.

#### P06.29

##### Molecular diagnosis of familial hypobetalipoproteinemia

R. Roig<sup>1</sup>, S. Martínez<sup>1</sup>, A. Beteta<sup>1</sup>, F. Blanco<sup>1</sup>

<sup>1</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

**Introduction:** Familial hypobetalipoproteinemia (FHBL) is a rare autosomal codominant disease characterized by < 5th percentile of the age-and sex-specific levels of LDL-cholesterol. Most cases are caused by mutations in the APOB gene and, less frequently, by ANGPTL3 mutations. Some FCHL patients with APOB mutations, but not with ANGPTL3 mutations, present with hepatic steatosis and intestinal lipid malabsorption. The molecular characterization of these entities is thus often needed for clinical diagnosis and genetic counseling.

**Aims:** To perform molecular diagnosis of FHBL in 20 suspected cases.

**Materials and Methods:** DNA was extracted from whole blood samples of 20 probands and subjected to PCR and Sanger sequencing of exons and flanking exon-intron of the APOB gene. Probands without APOB mutation were subjected to ANGPTL3 screening.

**Results:** From the 20 probands with the suspected disorder, 10 of them presented APOB mutations. Of these, 9 were in exons and 1 in a splicing zone. A prematurely stop codon was generated in all these probands thus encoding truncated apoB-100 proteins. Two of the 10 probands without APOB mutations presented mutations in ANGPTL3. One was a 5pb deletion (c.363\_367delCTAA) generating a prematurely stop codon that impairs ANGPTL3 function.

**Conclusions:** APOB gene mutations were found in the half of the analyzed probands while ANGPTL3 gene mutations were found in the 20% of probands without APOB gene mutation. The identification of unresolved cases could be improved by studying other genes potentially causing inherited hypolipidemia.

#### P06.31

##### In vitro expression analysis of eight phenylalanine hydroxylase variants: correlation with metabolic phenotypes and structural properties

R. Trunzo<sup>1</sup>, R. Santacroce<sup>1</sup>, G. De Girolamo<sup>1</sup>, A. Leccese<sup>1</sup>, M. Margaglione<sup>1</sup>, N. Blau<sup>2</sup>

<sup>1</sup>Laboratory of Human Genetics, Foggia, Italy, <sup>2</sup>Dietmar-Hopp Metabolic Center, Heidelberg, Germany

Hyperphenylalaninemias are genetic diseases prevalently caused by a wide range of mutations in phenylalanine hydroxylase (PAH) gene. In vitro expression analysis of mutations offers the opportunity to elucidate the molecular mechanisms involved, investigating the severity of biochemical phenotype, and how a mutation exerts its deleterious effects on the enzyme. To study the effects of mutations on PAH activity, we used an in vitro expression system in combination with liquid chromatography applied with ESI-MSMS for the quantification of tyrosine produced from phenylalanine to assess the residual activities of 8 missense variants (Y204C, L212P, L249P, L249F, R270K, R261P, S196Y and T380M) associated with PAH deficiency. The assays showed a decreased activity ranging from 7% to 51% that of the wild-type protein, with the exception of Y204C, which revealed no significant impact on enzyme function (94±6%). Three mutations (L249F, S196Y, and T380M) were associated with low-intermediate levels of activity (21-51%). Mutations L212P, L249P, R270K, and R261P showed very low residual activity (7-17%).

Moreover, in western blot analysis, all of the mutant proteins with rather

low activity (L212P, R270K, R261P) also presented with reduced amounts of protein. Instead, L249P, S196Y, T380M with low activity showed substantial amount of protein. In particular, L249P showed a clear contrast between strong reduction of activity (7±0,11) and high protein expression. Results from the experimental modulation of mutant residual activity have major implications, both for our understanding of genotype-phenotype correlations and for the discussion of molecular basis of BH4 responsiveness, useful for the development of novel therapeutic approaches.

#### P06.32

##### Targeted next generation sequencing in patients with inborn errors of metabolism

D. Yubero<sup>1</sup>, J. Armstrong<sup>2</sup>, A. García-Cazorla<sup>2</sup>, B. Perez-Dueñas<sup>2</sup>, F. Palau<sup>2</sup>, R. Artuch<sup>2</sup>, *Inborn Errors of Metabolism working group;*

<sup>1</sup>Fundació Sant Joan de Déu, Barcelona, Spain, <sup>2</sup>Hospital Sant Joan de Déu, Barcelona, Spain

**Introduction:** Next generation sequencing technologies (NGS) have allowed to promote a cheaper and faster genetic diagnosis. However, due to the uncertainties of NGS, the need of a holistic approach to inborn errors of metabolism (IEM) diagnosis is still unambiguous. To evaluate the NGS utility on the clinical field, targeted genic panel approach was designed for the diagnosis of a set of IEM. The aim of the work was to compare the diagnostic yield of NGS in patients presenting consistent clinical and biochemical suspicion of IEM with those with no specific biomarkers.

**Materials and methods:** Subjects studied (n=146) were classified in 2 categories: Group 1 (n=81), patients with consistent clinical and biochemical suspicion of IEM; Group 2 (n=65), cases with clinical suspicion of IEM but with unspecific or negative biomarkers. 175 genes (defects in amino acids, organic acids, free fatty acid oxidation, neurometabolic and complex molecules metabolism) were studied through HaloPlex Target Enrichment System and Illumina sequencing.

**Results:** Definitive genetic diagnosis was achieved in 73 out of the 146 patients (50%); in 12/146 (8.2%) only one mutation or variants with uncertain significance were detected in candidate genes; and in 61/146 (41.8%), no mutations were found. For group 1, the diagnostic yield was 78% (63 out of 81). This rate went down to 15.4% (10 out of 65) in group 2 (X<sup>2</sup> = 76.171; p < 0.0001).

**Conclusions:** Genetic diagnosis in our cohort was fast and effective, especially in the group having both clinical and biochemical indication for the diagnosis.

#### P06.33

##### Second tier next-generation sequencing for inborn errors of metabolism after positive newborn screening results.

E. Lundman<sup>1</sup>, A. Stray-Pedersen<sup>1</sup>, J. Strand<sup>1</sup>, J. Gaup<sup>1</sup>, T. Tangerås<sup>1</sup>, J. Jørgensen<sup>1</sup>, T. Rootwelt<sup>1,2</sup>, R. D. Pettersen<sup>1</sup>

<sup>1</sup>Oslo University Hospital, Oslo, Norway, <sup>2</sup>University of Oslo, Oslo, Norway

**Introduction:** Newborn screening (NBS) in Norway is mandated for 20 Inborn Errors of Metabolism (IEM). Rapid genotyping after a positive NBS result may have a major impact on follow-up and treatment. Using NBS screening samples we evaluated next generation sequencing using a large gene panel as 2nd tier tests for IEM.

**Materials and Methods:** Six samples with known diagnoses (1 PKU+TFF, 1 CPT2, 2 PA and 2 MMA) were included, 5 with known genotype. DNA (~1.8ng) extracted from the NBS dried blood spots was amplified with the IonAmpliSeq IEM panel (570 genes) and sequenced on IonTorrent PGM. Using Ion Reporter software, the detected variants were filtered to only show variants within the 32 relevant NBS-IEM genes, in exonic or splice regions, with 5000Exomes Global MAF<0.01.

**Results:** In total 1740-2122 variants were detected in each sample, with 2-12 variants remaining after initial filtering. In the 5 previously genotyped samples, all disease-causing variants were confirmed. In one MMA sample with unknown genotype, only three potential variants remained after filtering, including the two which likely account for the phenotype: NM\_052845.3(MMAB):[c.291-1G>A();c.571C>T]. The results were available within 3 days after DNA extraction.

**Conclusion:** The tested workflow may reduce turn-around-time for genotyping substantially, and show the possibility of quickly confirming positive metabolic screening even with large genes and multiple disease-genes involved. Bioinformatic filtering before variant evaluation avoids incidental findings outside the screening mandate genes and drastically shortens analysis time. A smaller gene panel may reduce costs and allow higher coverage of the gene variants of interest.

## P06.34

### A newly identified mitochondrial tRNA modification due to QRSL1 mutations causes infantile mitochondrial disease.

*N. Akiyama<sup>1</sup>, K. Murayama<sup>2</sup>, M. Shimura<sup>2</sup>, T. Fushimi<sup>2</sup>, K. Ichimoto<sup>1</sup>, M. Mori<sup>3</sup>, Y. Kishita<sup>4</sup>, Y. Tokuzawa<sup>4</sup>, M. Kohda<sup>4</sup>, T. Suzuki<sup>5</sup>, Y. Okazaki<sup>4</sup>, A. Ohtake<sup>6</sup>*

<sup>1</sup>Department of Clinical Genetics, Chiba Children's Hospital, Chiba, Japan, <sup>2</sup>Department of Metabolism, Chiba Children's Hospital, Chiba, Japan, <sup>3</sup>Department of Pediatrics, Matsudo City Hospital, Chiba, Japan, <sup>4</sup>Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan, <sup>5</sup>Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Tokyo, Japan, <sup>6</sup>Department of Pediatrics, Saitama Medical University, Saitama, Japan.

**Background:** Defects of tRNA modification (tRNA modopathy) are known as a cause of a variety of diseases. We present two cases of infantile mitochondrial disease with pathogenic mutations in QRSL1 (hGatA) involved in Glu-tRNAGln amidotransferase (Glu-Adt), which is composed of three subunits, hGatC, hGatA and hGatB.

**Patients and methods:** A girl (Pt250) suddenly suffered from hypoglycemia and lactic acidosis on day 1. Subsequently tachypnea, hypertrophic cardiomyopathy, adrenal insufficiency and hearing loss appeared. She died of cardiac failure at 5 months. Another girl (Pt860) developed cyanosis and lactic acidosis on day 3. She died of interstitial pneumonia at 2 months. We performed enzyme analysis, whole exome sequencing and their validation tests. **Results:** The enzyme analysis showed combined respiratory chain deficiencies (I, III, and IV) in both cases. The whole exome sequencing revealed Pt250 harbored a homozygous mutation c.398G>T (p.G133V) and Pt860 harbored a compound heterozygous mutation c.398G>T (p.G133V) and c.350G>A (p.G117E) in QRSL1. In vitro reconstitution of Gln-tRNAGln formation using recombinant hGatCAB showed strongly decreased transamidation activity.

**Conclusions:** Our study is the first to demonstrate that QRSL1 mutations lead to a defect in the human mitochondrial translation and are a cause of severe infantile mitochondrial disease.

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## P06.35

### ISCA2 mutation causes hereditary spastic paraparesis

*I. Dorboz<sup>1</sup>, F. Renaldo<sup>2</sup>, A. Slama<sup>3</sup>, J. Benoit<sup>2</sup>, M. Elmaleh<sup>2</sup>, V. Desportes<sup>4</sup>, O. Boespflug-Tanguy<sup>2</sup>*

<sup>1</sup>INSERM, Paris, France, <sup>2</sup>Robert Debré Hospital, Paris, France, <sup>3</sup>Bicêtre Hospital, AP-HP, Le Kremlin Bicêtre, Paris, France, <sup>4</sup>CHU Lyon, Hôpital Femme Mère Enfant, Bron, Lyon, France.

Iron-sulfur (Fe-S) clusters are ubiquitous cofactors composed of iron and inorganic sulfur. [Fe-S] clusters participate in electron transfer, substrate binding, iron, sulfur storage, regulation of gene expression, and enzyme activity. Various biogenesis components are functionally impaired leading to human disease. An early onset lethal encephalopathy has been recently described in relation to mutations of *ISCA2*. This protein is essential with *ISCA1* and *IBA57* for protein assembly required for [4Fe-4S]. We describe a new *ISCA2* mutation in a 12-year-old boy from a consanguineous Turkish family. He presented with a slowly progressive spastic paraparesia. At the age of 15 months he developed pyramidal signs in the lower limbs, however there was no dystonia or cerebellar signs. Brain MRI showed diffuse white matter abnormality, with areas of cavitation. Examination at the age of 8 years shows improvement in clinical signs but not in MRI with an extension of white matter abnormalities. Activities of the respiratory chain complexes were normal in fibroblasts whereas PDH activity was in the low range. Exome sequencing combined to homozygosity mapping revealed a homozygous mutation in *ISCA2* c.154C>T/p.Leu52Phe, parents were heterozygous for the variant. The mutation was predicted to be deleterious and was not found in the public databases. The reason for the clinical and biological differences between our patient and those previously described is not clear. However a variable phenotype expression was already described in other Iron-sulfer clusters proteins such as *IBA57* and *NFU1*, suggesting that other potential factors that might contribute variability in modulating phenotype

## P06.36

### A GWAS of first phase glucose stimulated insulin secretion during an intravenous glucose tolerance test reveals new physiology of genetic variants associated with type 2 diabetes

*N. van Leeuwen<sup>1</sup>, A. R. Wood<sup>2</sup>, A. Jackson<sup>3</sup>, A. Jonsson<sup>4</sup>, N. Wang<sup>5</sup>, U. Smith<sup>6</sup>, L. Boquete Vilarino<sup>2</sup>, A. Stancakova<sup>7</sup>, M. Walker<sup>8</sup>, R. Hanson<sup>9</sup>, T. M. Frayling<sup>2</sup>, Genome Utilisation for INtraveNous glucose stimulated insulin Secretion Studies GUINNESS consortium*

<sup>1</sup>LUMC, Leiden, Netherlands, <sup>2</sup>University of Exeter Medical School, Exeter, United Kingdom, <sup>3</sup>University of Michigan School of Public Health, Ann Arbor, MI, United States, <sup>4</sup>University of Copenhagen, Copenhagen, Denmark, <sup>5</sup>Keck School of Medicine of USC, Los Angeles, CA, United States, <sup>6</sup>University of Gothenburg, Gothenburg, Sweden, <sup>7</sup>University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland, <sup>8</sup>Newcastle University, Newcastle upon Tyne, United Kingdom, <sup>9</sup>The National Institute of Diabetes and Digestive and Kidney Diseases, Phoenix, AZ, United States.

**Introduction:** The mechanism underlying deterioration of insulin secretory capacity which plays an important role in the development of type 2 diabetes (T2D) is not fully understood. To identify genetic factors involved we meta-analyzed all studies where first phase insulin secretion has been investigated through intravenous glucose tolerance tests (IVGTT) and where GWAS (n=4834) or metabochip (n=741) data was available.

**Methods:** The traits Acute Insulin Response to glucose (AIR), Peak Insulin (PI) and Disposition Index (DI) were defined. Variants were imputed using the 1000G reference panel and tested for association using linear regression adjusted for age and sex and in an additional analysis also for BMI.

**Results:** A common T2D variant in *MTNR1B* was associated with the IVGTT traits at genome wide significance (PI p=2.5×10<sup>-26</sup>, AIR p=4.3×10<sup>-19</sup>, DI p=3.3×10<sup>-17</sup>) and variants in *CDKAL1* with both PI(p=4.9×10<sup>-15</sup>) and AIR(p=9.5×10<sup>-13</sup>), these variants also influenced oral glucose stimulated insulin secretion (OGTT). Other common T2D variants were associated at p<0.01 revealing potential mechanisms for these variants. Variants in *IGF2BP2* and *ADCY5* influenced the IVGTT but had little effect on the OGTT suggesting they exert direct effects on the pancreas rather than systemic effects. Putative novel associations were observed on chromosomes 7, 11 and 2 (p<5×10<sup>-8</sup>) but these variants need further validation.

**Conclusion:** Our genome wide analysis on the largest dataset for intravenously measured insulin secretion showed that T2D risk alleles at the *MTNR1B* and *CDKAL1* loci were strongly associated with our measures and it provides new insight into physiological mechanisms of other diabetes risk variants.

## P06.37

### The importance of both galactocerebrosidase enzyme analysis and *GALC* gene testing for diagnosis of Krabbe leucodystrophy

*D. Cregeen, E. Green, O. Chiu, M. Jackson; Viapath, Guy's Hospital, London, United Kingdom.*

Krabbe disease is a very rare autosomal recessive lysosomal storage disorder caused by deficiency of the enzyme galactocerebrosidase (galactosylceramidase). Traditionally diagnosis relied on measurement of enzyme deficiency using a radiolabelled natural substrate; a method in use at our laboratory for well over 30 years. Subsequently a more convenient fluorimetric substrate has been implemented in some laboratories, however there are known (albeit infrequent) issues with this methodology including false positive and negative results. Most laboratories are now using *GALC* gene analysis as a first or second line test for Krabbe disease. A recurrent 30kb *GALC* deletion has been identified in some populations however many *GALC* variants are family specific and therefore definitive enzyme analysis (either pre- or post- DNA testing) can be invaluable for confirmation of a diagnosis. Sanger sequencing of the *GALC* coding region has been carried out in 32 families at our centre to date. We present several patients with novel pathogenic variants and also several patients with a false positive enzyme deficiency caused by a non-pathogenic sequence variant (c.550C>T) which is thought to confer reduced enzyme activity *in vitro* (possibly more frequently observed with the fluorimetric enzyme methodology). The possibility of pseudodeficiency and the preponderance of novel variants in Krabbe patients highlights the importance of both galactocerebrosidase enzyme analysis and *GALC* gene testing for diagnosis of this disorder.

## P06.39

### Pharmacological intervention in Fabry disease and the role of proteostasis

*S. Seemann<sup>1</sup>, M. Ernst<sup>2</sup>, L. R. Trenkner<sup>1</sup>, G. Fuellen<sup>2</sup>, J. Lukas<sup>1</sup>, A. Rolfs<sup>1,3</sup>*

<sup>1</sup>Albrecht Kossel Institute, Medical University Rostock, Rostock, Germany, <sup>2</sup>Institute for Biostatistics and Informatics in Medicine and Ageing Research, Medical University Rostock, Rostock, Germany, <sup>3</sup>Centogene AG, The Rare Disease Company, Rostock, Germany.

Fabry disease is a lysosomal storage disorder resulting from a mutation in the gene encoding for the lysosomal enzyme  $\alpha$ -galactosidase A (GLA). These mutations result in a lack or a reduction of enzyme activity. Missense mutations often lead to a destabilized but still catalytically active enzyme. Small molecules have been shown to support protein folding and to reduce the re-

jection of the enzyme by the ER associated degradation, which consequently leads to enhanced transport to the lysosomes.

We identified small molecules that showed the capability to elevate the activity of mutant  $\alpha$ -galactosidase A in human fibroblasts from Fabry patients carrying the p.R301Q mutation. Also, synergistic effects of compounds could be shown arguing for a differential mode of action. In order to investigate more closely the specific mode of action of the proteostasis regulators we found to be effective, HTA-2.0 microarrays will be performed to compare transcriptomics data of treated and untreated p.R301Q fibroblasts. Resulting transcriptional fingerprints of the effective small molecules will be integrated and visualised on a proteostasis network based on biological function or physical interaction. To establish this network we reviewed several hundred proteostasis components with the help of commercial and non-commercial databases like Selventa and String.

We will finally identify sub-networks and single protein molecules within the proteostasis network as effective modulators of gene function and, perspective, as potential targets for a specific therapeutic intervention. The methodology is a generalizable tool to be transferred also to other lysosomal disorders and to identify new targets for treatment approaches.

## P06.41

### Identification of the functional miRNA/miRNAs in the pathogenesis of MODY3

O. F. Baltaci<sup>1</sup>, S. Colakoglu<sup>2</sup>, G. Gullu<sup>2</sup>, A. Karabay Korkmaz<sup>1</sup>, E. Berber<sup>3</sup>;

<sup>1</sup>Istanbul Technical University, Istanbul, Turkey, <sup>2</sup>Marmara University, Istanbul, Turkey,

<sup>3</sup>Istanbul Arel University, Istanbul, Turkey.

**Introduction:** MODY3 is a progressive hyperglycemia, associated with HNF1A gene. The HNF1A gene encoded transcription factor HNF1A regulates expression of genes including glucose metabolism and glucose transportation genes, insulin gene. MODY3 is misdiagnosed as Type1 Diabetes due to the similar clinical features. miRNAs are involved in the development of diabetes, diabetic complications, insulin expression. The present study explores the functional miRNAs in the pathogenesis of MODY3 by determining the HNF1A regulated miRNA/miRNAs in MIN6 cells.

**Materials and Methods:** Glucose stimulated MIN6 cells, expressing HNF1A endogenously, were transfected with the human HNF1A cDNA expression vector for overexpression and with siRNA binding to HNF1A mRNA to silence HNF1A expression. Overexpression and silencing were confirmed with RT-qPCR and Western Blotting. The HNF1A regulated miRNA/miRNAs was determined by RNA-Seq of the total RNA extract in the HNF1A overexpressed and silenced cells.

**Results:** 238 known and 91 novel miRNAs showed significantly differential expression when total RNA extract expression from HNF1A overexpressed and silenced cells were compared. Previously known miR-129-1-3p, miR-129-2-3p, miR-200b-3p, miR-296-3p, and miR-378a-5p, associated with diabetes in the literature, showed the most significant differential expression.

**Conclusion:** This study revealed presence of HNF1A regulated known and novel miRNAs in MIN6 cells. The most significantly differentially expressed 5 miRNAs are selected as candidate miRNA molecules in the pathogenesis of MODY3. Validation of the candidate miRNAs and their effect on insulin secretion in MIN6 cells are in progress. Further studies in the serum samples of MODY3 patients will reveal their role as a biomarker in the diagnosis.

## P06.42

### Functional Characterization of p.T10M and p.S345K mutations in HNF1A Gene in MODY patients

O. Yalcin Capan<sup>1</sup>, O. F. Baltaci<sup>1</sup>, E. Selcuk<sup>2</sup>, E. Berber<sup>1</sup>;

<sup>1</sup>Istanbul Arel University, Istanbul, Turkey, <sup>2</sup>Istanbul Technical University, Istanbul, Turkey.

**Introduction:** Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterized by abnormal beta cell function, autosomal dominant inheritance, hyperglycemia, lack of auto-immunity in non-obese young patients. MODY3 associated with HNF1A gene mutations is the most common form of MODY. HNF1A is a transcription factor that regulates a number of liver-specific genes and genes involved in glucose metabolism. **Materials and Methods:** The functional properties of HNF1A gene variations (p.T10M, p.S345K) which were identified in Turkish MODY patients were analyzed. The effects of the candidate mutations on HNF1A transactivation function were determined by dual luciferase reporter assay. The effects of mutations on the nuclear localization of HNF1A were analyzed by immunofluorescence confocal microscopy. DNA binding activity of mutant HNF1A and wild type proteins were also compared by colorimetric DNA-protein binding assay.

**Results:** Dual luciferase assay results showed that both p.T10M and p.S345K mutant proteins have similar transactivation activity as the wild type HNF1A. Immunostaining studies revealed that p.T10M mutant protein localize in the nucleus as the wild type HNF1A while p.S345K mutant protein is in the cytoplasm. DNA binding ability of p.T10M mutant protein was reduced, but p.S345Y mutant similar compared to wild type.

**Conclusion:** Preliminary results showed that both mutant HNF1A proteins have reduced activities and malfunctions compared to wild type. Future studies will show whether they also effect the insulin secretion from pancreatic cells. This project (113S218) was supported by TÜBİTAK (The Scientific and Technical Research Council of Turkey).

## P06.43

### Reprogramming of patients-derived fibroblasts to pluripotency for modeling inherited disorders of propionate metabolism

E. Richard, S. Brasil, M. Ugarte, L. R. Desviat, B. Pérez;  
Universidad Autónoma de Madrid, Madrid, Spain.

Recent advances in induced pluripotent stem cell (iPSC) technologies have transformed our ability to elucidate the pathogenic mechanisms of disease development and to test new therapeutic strategies. iPSCs are capable of self-renewal and have the potential to differentiate into any cell type which can now help to overcome the limitations of fibroblasts as cellular model for inherited metabolic disorders. This work has focused on the generation and characterization of iPSCs from two patients-derived fibroblasts with methylmalonic aciduria cblB type (MIM #251110) and one with propionic acidemia PCCA type (MIM #606054) using CytoTune Sendai vectors which include the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc). The iPSCs generated have been characterized and presented the hallmarks for these cells: (1) typical iPSC-like morphology and growth characteristics, (2) conservation of the mutations identified in the three patients, (3) positive staining for alkaline phosphatase activity, (4) expression of pluripotency-associated markers (OCT4, NANOG and SOX2) and surface markers (SSEA3, SSEA4, TRA1-60, and TRA1-81), (5) demethylation of the OCT4 and NANOG promoters, (6) normal karyotype and (7) differentiation potential into all three primary germ cell layers in vitro. Our next step will be the iPSC differentiation into neurons, hepatocytes and cardiomyocytes serving as an in vivo platform for modeling these two disorders of propionate metabolism.

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## P06.44

### Functional characterization of four novel genetic variants causing methylmalonic aciduria and propionic academia in Serbian patients

M. Stojiljkovic<sup>1</sup>, K. Klaasen<sup>1</sup>, M. Djordjevic<sup>2</sup>, A. Sarafijija<sup>2</sup>, S. Brasil<sup>3</sup>, B. Kecman<sup>2</sup>, S. Grkovic<sup>2</sup>, J. Kostic<sup>1</sup>, P. Rodriguez-Pombo<sup>3</sup>, L. R. Desviat<sup>3</sup>, S. Pavlovic<sup>1</sup>, B. Perez<sup>2</sup>;

<sup>1</sup>IMGGE, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic“, Belgrade, Serbia, <sup>3</sup>Centro de Diagnóstico de Enfermedades Moleculares, CIBERER, Madrid, Spain.

Methylmalonic aciduria (MMA) and propionic acidemia (PA), inborn metabolic diseases inherited in an autosomal-recessive manner, are the most frequent organic acidurias. However, each of them is classified as rare disease since their incidences are estimated at ~ 1:50 000 for MMA and ~ 1:100 000 -150 000 for PA. Specific mitochondrial enzymatic deficiencies in the catabolism of branched-chain amino acids cause MMA and PA.

Four MMA and one PA patients from Serbia were analyzed. We detected five previously described variants: p.Asn219Tyr, p.Arg369His p.Val553Glyfs\*17 in *MUT*, p.Thr198Serfs\*6 in *MMAA*, p.Ile144\_Leu181del in *PCCB* gene and four novel genetic variants: p.Leu549Pro, p.Glu564\*, p.Leu641Pro in *MUT* and p.Tyr206Cys in *PCCB* gene.

*In silico* and eukaryotic expression studies confirmed pathogenic effect of all novel variants. Aberrant enzymes p.Leu549Pro *MUT*, p.Leu641Pro *MUT* and p.Tyr206Cys *PCCB* did not show residual activity in activity assays. In addition, activity of *MUT* enzymes was not rescued in the presence of vitamin B12 precursor *in vitro* which was in accordance with non-responsiveness or partial responsiveness of patients to vitamin B12 therapy.

Our study brings the first molecular genetic data and phenotypic characteristics for MMA and PA patients for Serbia and the whole South-Eastern European region. Therefore, our study contributes to the better understanding of molecular landscape of MMA and PA in Europe and to general knowledge on genotype-phenotype correlation for these rare diseases.

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## P06.45

### Mutations in the complex I assembly factor TMEM126B result in muscle weakness associated with complex I deficiency

**B. Ruzzene**<sup>1</sup>, **L. M. Sanchez**<sup>2</sup>, **L. Bianchi**<sup>1</sup>, **Z. Assouline**<sup>3</sup>, **M. D. Metodiev**<sup>4</sup>, **M. Rio**<sup>3</sup>, **B. Funalot**<sup>4</sup>, **A. M. Marié**<sup>1</sup>, **M. Van den Brand**<sup>2</sup>, **S. Guerrero-Castillo**<sup>2</sup>, **J. Molenaar**<sup>5</sup>, **D. Koolen**<sup>6</sup>, **U. Brandt**<sup>2</sup>, **R. J. Rodenburg**<sup>2</sup>, **L. G. Nijtmans**<sup>2</sup>, **A. Rötig**<sup>1</sup>;

<sup>1</sup>INSERM U1163, Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Paris, France, <sup>2</sup>Nijmegen Centre Mitochondrial Disorders, Dept Pediatrics, Radboud Univ Medical Centre, Nijmegen, Netherlands, <sup>3</sup>Departments of Pediatric, Neurology and Genetics, Hôpital Necker-Enfants-Malades, Paris, France, <sup>4</sup>Department of Genetics, Henri Mondor Hospital, Créteil, France, <sup>5</sup>Department of Neurology, Radboud University Medical Center, Geert Grootplein Zuid 10, Nijmegen, Netherlands, <sup>6</sup>Department of Human Genetics, Radboud University Medical Center, Geert Grootplein Zuid, Nijmegen, Netherlands.

Mitochondria are the powerhouses of all eukaryotic cells which produce energy in a process called oxidative phosphorylation (OXPHOS). Complex I (CI), the first and the biggest of five multi-protein complexes that compose the mitochondrial OXPHOS system, consists of both mitochondrial and nucleus-encoded subunits. Because of its size and its dual genetic origin, biogenesis of CI is a complex event that requires a set of not yet completely characterized assembly factors. Mutations in genes encoding either CI structural subunits or assembly factors result in isolated CI deficiency, which represents the most common cause of OXPHOS dysfunction.

Using whole exome sequencing and targeted exome sequencing, we identified *TMEM126B* as a disease-causing gene in three unrelated patients presenting with exercise intolerance and muscle weakness due to isolated CI deficiency. *TMEM126B* is a putative CI assembly factor that is not well characterized and its mutations led to the accumulation of CI assembly intermediates in patient fibroblasts. Lentiviral complementation with the wild-type cDNA restored the assembly of CI confirming the pathogenic nature of the identified mutations and establishing *TMEM126B* as a novel mitochondrial disease gene.

## P06.46

### Mitochondrial dysfunction and aberrant methylation in nuclear genes

**L. Mayorga**<sup>1</sup>, **S. Laurito**<sup>1</sup>, **E. Campoy**<sup>1</sup>, **H. Eiroa**<sup>2</sup>, **M. Roqué**<sup>1</sup>;

<sup>1</sup>Institute of Histology and Embryology CONICET National University of Cuyo, MENDOZA, Argentina, <sup>2</sup>IP Garrahan Children's Hospital, Buenos Aires, Argentina.

Introduction: It is known that environment has an impact on epigenomes, especially of cancer cells. It is, however, not clear how this occurs. A hallmark of cancer cells is energy reprogramming whereby glycolysis is preferred over OXPHOS. We propose that mitochondria might play a role in epigenome modifications. The aim of this study was therefore to investigate the role of mitochondrial dysfunction in aberrant methylation of nuclear genes. Methods: Methylation analyses of 26 nuclear genes were performed by MS-MLPA on: -leucocytes and muscle samples from 23 mitochondrial disease patients; -human skeletal muscle (HSkM) cell line treated with Rotenone, an OXPHOS inhibitor. OXPHOS function was determined by Trimethylrodamine ester stain and quantifications on confocal microscopy were performed using ImageJ. Methylation was analyzed by GeneMarker v1.95.

Results: muscle samples from patients presented higher frequency of aberrantly methylated genes than leucocytes ( $p=0.02$ ). Caspase8 was the most frequently methylated gene in muscle (76,9% vs 0% in leucocytes). Unexpectedly, experiments in OXPHOS inhibited HSkM cells revealed changes in the methylation of a single gene: Caspase8. The methylation status of this gene was augmented when the mitochondrial membrane potential came down due to Rotenone action and reverted when Rotenone was removed.

Conclusions: Our observations indicate that mitochondria's dysfunction is related to methylation alterations in nuclear genes. These epigenetic findings could contribute in the practice, to explain the wide phenotypic spectrum of mitochondrial diseases. In addition, this suggests that mitochondria have a potential role in the link between environment and epigenome.

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## P06.47

### New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies

**A. Legati**<sup>1</sup>, **A. Reyes**<sup>2</sup>, **A. Nasca**<sup>1</sup>, **F. Invernizzi**<sup>1</sup>, **E. Lamantea**<sup>1</sup>, **B. Garavaglia**<sup>1</sup>, **C. Lamperti**<sup>1</sup>, **A. Ardissono**<sup>3</sup>, **I. Moroni**<sup>3</sup>, **A. Robinson**<sup>2</sup>, **D. Ghezzi**<sup>1</sup>, **M. Zeviani**<sup>4</sup>;

<sup>1</sup>Unit of Molecular Neurogenetics - Fondazione IRCCS Istituto Neurologico 'Carlo Besta', Milan, Italy, <sup>2</sup>Mitochondrial Biology Unit - Medical Research Council, Cambridge, United Kingdom, <sup>3</sup>Unit of Child Neurology - Fondazione IRCCS Istituto Neurologico 'Carlo Besta', Milan, Italy, <sup>4</sup>Mitochondrial Biology Unit - Medical Research Council, Milan, Italy.

Next Generation Sequencing (NGS) technologies are revolutionizing the diagnostic and research screening for rare diseases, particularly for those genetically and clinically heterogeneous like primary mitochondrial disorders. NGS approaches are particularly suitable for investigating the causative mutations in small families and even in single individuals, for which the traditional linkage analysis is limited. These technologies, in fact, are contributing to deepen the knowledge on the heterogeneous genetic causes of mitochondrial diseases and to significantly reduce the percentage of cases lacking a molecular diagnosis, with several new disease genes discovered. In this study we analyzed a cohort of 125 patients, that failed to show mutations in mtDNA and in specific nuclear genes after traditional Sanger's sequencing, performing a combined, two-step strategy, based on targeted genes panel as a first NGS screening, followed by whole exome sequencing (WES) in still unsolved cases. This approach has allowed us to reach a molecular diagnosis in the 20% of these difficult cases, but it has also revealed unexpected and conceptually new findings. These include the possibility of marked variable penetrance of recessive mutations, the identification of large-scale DNA rearrangements, which explain spuriously heterozygous cases, and the association of mutations in known genes with clinical phenotypes never described before. Importantly, WES on selected cases allowed us to discover pathogenic mutations in genes encoding non-mitochondrial proteins, an observation that widens the complex genetic heterogeneity of mitochondrial disease and suggests a new area of investigation in mitochondrial medicine.

## P06.48

### Exome sequencing in infants with monogenic diabetes mellitus in Russia

**O. S. Glotov**<sup>1,2,3</sup>, **A. S. Glotov**<sup>1,2</sup>, **E. A. Zukova**<sup>1,2</sup>, **E. B. Bashnina**<sup>4</sup>, **O. S. Beresneva**<sup>4</sup>, **M. E. Turkunova**<sup>4</sup>, **I. V. Polyakova**<sup>1,5</sup>, **T. E. Ivaschenko**<sup>2</sup>, **S. V. Apalko**<sup>5</sup>, **M. A. Fedyakov**<sup>5</sup>, **N. Y. Shwed**<sup>1,2,5</sup>, **E. S. Shabanova**<sup>2</sup>, **D. Polev**<sup>1</sup>, **T. A. Dubinina**<sup>6</sup>, **L. V. Tyrtova**<sup>4</sup>, **M. A. Polyanskaya**<sup>4</sup>, **N. N. Lobanova**<sup>4</sup>, **A. S. Olenev**<sup>4</sup>, **V. V. Platonov**<sup>6</sup>, **T. E. Korytko**<sup>4</sup>, **A. V. Karpushkina**<sup>2</sup>, **V. S. Baranov**<sup>1,2</sup>;

<sup>1</sup>Saint Petersburg State University, St-Petersburg, Russian Federation, <sup>2</sup>FSBSI „The Research Institute of Obstetrics, Gynecology and Reproductology named after „D.O. Ott”, St-Petersburg, Russian Federation, <sup>3</sup>City Hospital №40, St.Petersburg, Russian Federation, <sup>4</sup>North-Western State Medical University named after I.I.Mechnikov, St-Petersburg, Russian Federation, <sup>5</sup>City Hospital №40, St-Petersburg, Russian Federation, <sup>6</sup>Children's Municipal Hospital №19 after K.A. Rauhfus, St-Petersburg, Russian Federation, <sup>7</sup>CAF Foundation, Moscow, Russian Federation.

Improvement of medical care by implementation of new molecular technologies is one of the major challenges of healthcare in the Russia. This approach is of special importance for molecular-genetic analysis of children with monogenic endocrine pathology as well as members of their families. NGS diagnostic panel of exon amplification of candidate genes involved in pathogenesis of hyperinsulinism and MODY was developed. It included the following candidate - genes: HNF1A, GCK, HNF4A, HNF1B, PDX1, NEUROD1, KLF11, CEL, PAX4, INS, BLK, EIF2AK3, RFX6, WFS1, ZFP57, FOXP3, KCNJ11, ABCC8, GLUD1, HADH (SHAD), SLC16A1, UCP2, INSR, AKT2, GCG, GCGR, PPARG, PTF1A. NGS was carried out for 21 patients by „HiSeq 2500“ („Illumina“, USA). The results of DNA sequencing were used for bioinformatic filtering with the following available programs: „GeneTalk“, „destroyed“, „reporter ion“, „to sift“, „PolyPhen2“, „PAPI“. Verification of the final results was done by direct Sanger sequencing with ABI3130 genetic analyzer. Pathogenic mutations and thus molecular-genetic diagnosis were so far confirmed only in 3 families. The mutations identified so far were in heterozygous state of GCK gene and included: c.772C>A; c. 199C>A; c.754A>G. According to our data MODY mutations distribution in Russian population seems to be rather similar to this one for other population. Identified mutations proved molecular basis of the disease, and might be useful for understanding the mechanism of disease and its potentially more efficient treatment.

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**P06.49****Novel variants in BCKDHA and BCKDHB genes cause maple syrup urine disease syndrome in Serbian patients**

**K. Klaassen<sup>1</sup>, M. Stojiljkovic<sup>1</sup>, M. Djordjevic<sup>2</sup>, A. Sarajlija<sup>2</sup>, S. Brasil<sup>3</sup>, B. Kecman<sup>2</sup>, S. Grkovic<sup>2</sup>, J. Kostic<sup>1</sup>, P. Rodriguez-Pombo<sup>3</sup>, L. R. Desviat<sup>3</sup>, B. Perez<sup>3</sup>, S. Pavlovic<sup>1</sup>**

<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic“, Belgrade, Serbia, <sup>3</sup>Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular, Universidad Autónoma de Madrid, CBMSO, UAM, CIBERER, Madrid, Spain.

Maple syrup urine disease syndrome (MSUD) is a rare metabolic disease resulting from deficient function of the branched-chain alpha-ketoacid dehydrogenase complex (BCKD), responsible for degradation of branched-chain amino acids leucine, isoleucine and valine. BCKD is composed of four components, E1a, E1b, E2, and E3, whereas variants in genes coding for E1a (*BCKDHA*), E1b (*BCKDHB*) and E2 (*DBT*) lead to MSUD (MIM# 248600).

In this study, we analyzed 4 Serbian patients diagnosed with MSUD according to biochemical data and clinical symptoms. We used Sanger sequencing for targeted analysis of *BCKDHA*, *BCKDHB* and *DBT* genes. In *BCKDHA* gene, we detected two previously described variants, c.1312T>A (p.Tyr438Asn) and c.861\_868delAGCCCCCG (p.Gly288Valfs\*11), and also two novel missense variants, c.581A>G (p.His194Arg) and c.892G>A (p.Val298Met). In *BCKDHB* gene, we detected one previously described variant, c.410C>T (p.Ala137Val), and a novel deletion, c.857\_871del15 (p.Glu286\_Met290del). All novel missense variants were predicted to be damaging by PolyPhen2, SIFT, PROVEAN and MutPred algorithms, and the protein sequence alignment using Clustal Omega pointed out to evolutionary conservation of affected residues. Protein modeling using I-TASSER showed that both novel *BCKDHA* variants lead to improper folding of the protein and thus result in an unstable protein.

This study provided the first data about molecular genetics of Serbian patients presenting with MSUD clinical symptoms, thus enabling molecular genetic diagnostics and genetic counseling of this disease in the country.

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**P06.52****Massive parallel sequencing-based genetic confirmation of metabolic disorders detected by the neonatal screening program**

**R. Navarrete, F. Leal, A. Vega, C. Medrano, I. Bravo-Alonso, L. R. Desviat, P. Rodriguez-Pombo, B. Merinero, M. Ugarte, C. Pérez-Cerdá, B. Pérez**

*Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular, Universidad Autónoma de Madrid, CIBERER, IdiPAZ, Madrid, Spain.*

The main purpose of expanded newborn screening (NBS) is to diagnose genetic disorders at an earlier step to start treatment before the appearance of clinical symptoms. In metabolic disorders, quantification of amino acids and acylcarnitines in dried blood spots by tandem mass spectrometry allows the detection of more than 30 different pathologies. The entire diagnosis process requires further sample collection for a second-tier test and genetic confirmation. In this work, we present our experience with the use of massive parallel sequencing (MPS) for genetic analysis of 84 consecutive DNA samples from newborns referred to confirm the results of the Spanish NBS program. The analysis was done by a customized panel to capture the sequence of the more frequent actionable metabolic disorders. Additionally, Illumina clinical-exome TruSightOne® combined with a gene virtual capture was used. Biallelic pathogenic mutations were found in 53 patients while only one mutation was detected in 24, in PAH, ACADM, ACADVL, GCDH, MCCC1, MCCC2, and SLC22A5. No mutations were found in some newborns with hyperphenylalaninemia or biochemical suspicion of VLCADD or GCDH deficiency. Pathogenic mutations (loss-of-function or previously described mutations) were found in 128 alleles while variants of unknown clinical significance were detected in 18 alleles. In two newborns suspected to have a variant condition of MSUD and argininemia, pathogenic mutations were detected for the first time in BCAT2 and SLC7A1 respectively. The results show the usefulness of MPS as second-tier test for diseases confirmation and for differential diagnosis of metabolic disorder. Grant: PI13/01239

**P06.53****Exome analysis in consanguineous families with neonatal diabetes suggests novel aetiological genes and new insights into beta-cell function**

**E. De Franco, J. A. L. Houghton, H. Lango Allen, S. E. Flanagan, C. Shaw-Smith, M. Wakeling, T. Laver, A. T. Hattersley, S. Ellard**  
University of Exeter Medical School, Exeter, United Kingdom.

A genetic diagnosis of neonatal diabetes (NDM) guides clinical management and can lead to improved treatment. Approximately 15% of patients born to consanguineous parents don't have mutations in known genes. The aim of this study was to identify novel recessive causes of NDM to gain insights into mechanisms underlying pancreatic beta-cell development and function. We performed exome sequencing and autozygosity mapping in 10 patients with NDM born to consanguineous parents in whom the known causes had been excluded.

We identified homozygous likely pathogenic variants in 5/10 patients. Two genes, *TMEM16A* and *CD274*, have not previously been linked to human disease. *TMEM16A* encodes a protein important for vesicular transport and interacts with the product of a known NDM gene (*JER3IP1*). *CD274* is essential for immune regulation and the knockout mouse phenotype supports an aetiological role. Three patients had mutations in previously reported disease genes: *EPG5* (regulates autophagy), *SPATA5* and *COQ9* (both important for mitochondrial function). Importantly for our patient, individuals with *COQ9* mutations can be successfully treated with oral CoQ10 therapy. In addition to NDM, these three patients had clinical features overlapping previously reported cases. Although the possibility of a dual aetiology cannot be excluded, these genes encode proteins in pathways known to be affected in the pathophysiology of diabetes, supporting their role in NDM. Identification of further patients is required to confirm these findings.

In conclusion, our approach was highly successful finding the likely genetic diagnosis in 5/10 patients and highlighting the possible role of five genes in beta-cell function/development.

**P06.54****Production of induced Pluripotent Stem cells (iPSC) from patients with „neutral lipid storage disease with myopathy“: perspectives for a model of disease in vitro**

**D. Degiorgio<sup>1,2</sup>, S. Missaglia<sup>3</sup>, M. Castagnetta<sup>1</sup>, E. M. Pennisi<sup>4</sup>, M. Mogni<sup>1</sup>, D. A. Coviello<sup>1</sup>, D. Tavian<sup>3,5</sup>**

<sup>1</sup>Laboratory of Human Genetics, E.O. Ospedali Galliera, Genoa, Italy, <sup>2</sup>Department of Experimental Medicine, University of Genoa, Genoa, Italy, <sup>3</sup>Laboratory of Cellular

Biochemistry and Molecular Biology, CRIBENS, Catholic University of the Sacred Heart, Milan, Italy, <sup>4</sup>Department of Neurology, San Filippo Neri Hospital, Rome, Italy,

<sup>5</sup>Psychology Department, Catholic University of the Sacred Heart, Milan, Italy.

Mutations in PNPLA2 gene causes the „Neutral Lipid Storage Disease with Myopathy“, a very rare disorder characterized by a defect in the degradation of cytoplasmic triglycerides and accumulation as lipid droplets (LDs). This lipid dysmetabolism may determine progressive myopathy (100%), cardiomyopathy (44%), diabetes (24%), hepatomegaly (20%), chronic pancreatitis (14%) and short stature (15%). No specific therapy is available today. The fibroblasts from 2 patients and 1 healthy subject have been reprogrammed into induced pluripotent stem cells (iPSCs), through infection using the Sendai viral reprogramming kit. Both patients have a homozygous PNPLA2 mutation: in the first one the c.541\_542delAC causes a prematurely truncated protein, whereas in the second one the c.662G>C determines the amino-acid change p.(R221P). The iPSC were initially selected by their morphology and the expression of the enzyme alkaline phosphatase. We demonstrated their pluripotency properties evaluating by immuno-staining the expression of markers TRA-1-81, SSEA4 and OCT4, and their in vitro differentiation into three-germ layers (β-III tubulin, ectoderm; SMA, mesoderm; FOXA2, endoderm). The comparative gene expression profiles (by qRT-PCR) of SOX2 and NANOG in iPSC and their corresponding fibroblasts, shows the preferential gene expression in iPSC clones. The evaluation of additional genes (ZFP42, OCT4, hTERT, LIN28, DPPA2 and TDGF1) is ongoing. The immunohistochemical evaluation showed the presence of LDs in fibroblasts and also in iPSC of the patients. The perspective to differentiate iPSCs into striatum/cardiac muscle lineages, will allow us to define a disease model to investigate the pathogenetic mechanisms and to evaluate specific approaches for new pharmacological treatments.

## P06.55

## NGS - promises and obstacles in study of patients with inborn errors of metabolism

R. V. Vazharova<sup>1,2</sup>, L. Balabanski<sup>2</sup>, S. Ivanov<sup>2</sup>, S. Andonova<sup>3</sup>, M. B. Ivanova<sup>3,4,1</sup>, I. Sinigerska<sup>3</sup>, I. Bradinova<sup>3</sup>, S. Josifovska<sup>5</sup>, D. Avdjieva-Tzavella<sup>6</sup>, S. Hadjidekova<sup>7</sup>, A. Savov<sup>3</sup>, M. Malinov<sup>2</sup>, D. Toncheva<sup>7,2</sup>,

<sup>1</sup>Sofia University St. Kliment Ohridski, Sofia, Bulgaria, <sup>2</sup>GARH Malinov, Genomic Laboratory, Sofia, Bulgaria, <sup>3</sup>UHOG Maichin dom, National genetic laboratory, Medical University Sofia, Sofia, Bulgaria, <sup>4</sup>Sofia University „St. Kl. Ohridski“, Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, Sofia, Bulgaria, <sup>5</sup>Faculty of Natural Sciences, Ss. Cyril & Methodius University- Skopje, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>6</sup>Department of Clinical Genetics, University Pediatric Hospital, Medical University Sofia, Sofia, Bulgaria, <sup>7</sup>Department of Medical Genetics, Medical University - Sofia, Sofia, Bulgaria.

**Introduction:** Inborn errors of metabolism (IEM) constitute a large group of monogenic disorders disrupting metabolic pathways. Clinical presentation varies widely - from early onset (and sometimes fatal) disease in the newborn to progressive chronic form with onset in adult life. Massive parallel sequencing technology has the potential to uncover causative genetic variants in affected individuals and gives clues for their proper therapy.

**Materials and methods:** NGS targeted resequencing of large gene panels was used for genetic profiling of 13 probands with suspected IEM and healthy parents in a family where the proband was not available for DNA analysis. Nine patients presented typical symptoms and / or specific metabolic profile; others were with unclear or insufficient clinical data.

**Results:** In 7 affected probands causative gene mutations were found (data shown in table). Sanger sequencing was performed for confirmation of the results.

**Conclusion:** NGS of focused gene panels gives high diagnostic yield when applied to well-defined patient groups. Identification of causative mutations is possible even in families with missing probands and may help for their appropriate genetic counseling and medical management. Despite this, about 40% of patients may need WES/WGS to reveal a diagnosis.

Results from NGS genotyping					
Patient	Gene	Mutation 1	Mutation 2	Clinical observation	Inheritance
1	HADHA	p.Glu510Gln	p.Arg291Ter	newborn with cardiomyopathy and suspected Pompe disease	Autosomal recessive
2	HSD17B4	p.Gly16Ser	p.Gly16Ser	newborn with Zellweger spectrum disease	Autosomal recessive
3	NDUFAF6	p.Leu186_Tyr187delinsHis p.Gln99Argfs	p.His269Tyr	patient with Leigh syndrome	Autosomal recessive
4	NPC1	p.Thr1043Ala		child with Niemann-Pick disease	Autosomal recessive
5	BCKDHA	p.Glu327Lys	p.Glu327Lys	patient with MSUD	Autosomal recessive
6	PCCA	c.1209+3A>G	c.1209+3A>G	patient with Propionic acidemia	Autosomal recessive
7	PHKG2	p.Asp153Val	p.Leu160del	child with Glycogenosis	Autosomal recessive
8	ABCB11	p.Leu1126Ter	-	parents tested; child with Progressive intrahepatic cholestasis was unavailable for DNA analysis	Autosomal recessive

## P06.56

## Next Generation Sequencing: a good standard for mitochondrial disorders diagnosis

G. Grieco<sup>1</sup>, M. Valente<sup>1</sup>, S. Brandini<sup>2</sup>, V. Battaglia<sup>2</sup>, A. Olivieri<sup>2</sup>, S. Gagliardi<sup>1</sup>, I. Ricca<sup>1</sup>, S. Zucca<sup>1</sup>, A. Torroni<sup>2</sup>, C. Cereda<sup>1</sup>;

<sup>1</sup>Center of Genomics and post-Genomics, C. Mondino National Institute of Neurology Foundation, Pavia, Italy, <sup>2</sup>Department of Biology and Biotechnology, „L. Spallanzani“ University of Pavia, Pavia, Italy.

**Background:** Mitochondrial diseases are a group of clinically and genetically heterogeneous disorders with variable penetrance, expressivity, and different age of onset. Most disease-causing mtDNA mutations are heteroplasmic and the degree of heteroplasmy varies in different tissues. Thus, the determination of mutant loads in affected tissues is important in making the diagnosis and correlating clinical phenotype. Next Generation System (NGS) approach also allows simultaneous analyses of a group of genes or of the whole exome, thus, the mutations in causative gene(s) can be identified in one-step.

**Objective:** Aim of this study was to make genetic analysis in a much faster and more efficient way; and to identify the specific degree of heteroplasmy.

**Methods:** Nextera XT Illumina platform was used and NGS analysis has been performed on 52 DNA blood samples.

**Results:** Data analysis confirmed the classical mutation 8344R in a sample used as a positive control and showed the presence of risk factors as 7080,

13889 and 12634. The most interesting data concerned a sample which, with the restriction fragment length polymorphism (RFLP) methodology, did not show the presence of the 3243R mutation which, on the other hand, was observed at 13.6% with NGS technology.

**Discussion:** Our results confirmed that NGS was more sensitive and specific respect to other methods to study mtDNA. The use of deep sequencing allowed to identify both low-level of heteroplasmy and the presence of rare mutations.

## P06.57

## Descriptive report of the adult only-visceral phenotype of Niemann-Pick disease type C.

L. López de Frutos<sup>1,2</sup>, P. Alfonso<sup>1,2,3</sup>, J. J. Cebolla<sup>1,2</sup>, P. Irún<sup>1,2,3</sup>, P. Giraldo<sup>1,2,3</sup>,

<sup>1</sup>Spanish Foundation of Gaucher Disease and Other Lysosomal Disorders (FEETEG), Zaragoza, Spain, <sup>2</sup>Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain.

Niemann-Pick C disease (NPC) is a rare autosomal recessive lipid storage disorder with a heterogeneous presentation. It is characterized by visceral, neurological and psychiatric manifestations. Most of patients develop symptoms in early childhood; however, several phenotypes were identified in adults. The adult only-visceral phenotype is uncommon nevertheless is referred in some publications.

This study reports adult probands state NPC only-visceral phenotype and its characterization. Firstly, plasma biomarkers as Chitotriosidase activity (ChT), CCL18/PARC and 7-Ketocholesterol (7-KC) concentrations were assessed through biochemistry approaches. Lastly, genetic characterization of *NPC1* and *NPC2* were carried out.

In the last two years, 146 probands were recruited. 36 of them were identified at least one genetic variation reported as pathogenic or unknown significance (VUS) in NPC, 8/36 showed only-visceral symptoms; 3 of them were children who were excluded from the study. Focused in this 5 adult visceral cases, they were 38(27-47) [mean(min-max)] years old. ChT was 119(13-653) nmol/mL/hour. CCL18/PARC and 7-KC concentrations were 157(65-178) and 210.5(>2-333)ng/mL respectively. Variants identified in *NPC1*, reported as pathogenic or VUS, were: p.C177T, c.463+19A>G, p.Q775P, p.N916S, p.I1061T and p.A1151T. *NPC2* gene does not show any variations. All genetic variations were found in heterozygosity and only one subject presented two variants.

Those results underline the need to perform complementary studies to evaluate the possible penetrance of some variations inherited in heterozygosity. It is necessary to analyze more patients with this only-visceral phenotype to establish some relationship between this form and some genetic variants.

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## P06.58

## Microarray expression profile analysis in Niemann-Pick type C fibroblasts: preliminary results of a pilot study

K. Hetmanczyk<sup>1</sup>, R. Iwanicka-Nowicka<sup>2,3</sup>, A. Fogtman<sup>2</sup>, M. Koblowska<sup>2,3</sup>, M. Filocamo<sup>4</sup>, S. Lualdi<sup>4</sup>, B. Żyłyńska-Granica<sup>5</sup>, P. Włodarski<sup>6</sup>, A. Ługowska<sup>1</sup>;

<sup>1</sup>Department of Genetics, Institute of Psychiatry and Neurology Institute of Psychiatry and Neurology, Warsaw, Poland, <sup>2</sup>Laboratory of Microarray Analysis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland, <sup>3</sup>Laboratory of Systems Biology, Faculty of Biology, University of Warsaw, Warsaw, Poland, <sup>4</sup>Centro di Diagnostica Genetica e Biochimica delle Malattie Metaboliche, Istituto G. Gaslini, Genova, Italy, <sup>5</sup>Department of Pharmacodynamics, Medical University of Warsaw, Warsaw, Poland, <sup>6</sup>Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland.

**Introduction:** Niemann-Pick type C (NPC) disease is a rare lysosomal disorder due to mutations in the genes *NPC1* or *NPC2*. Proteins NPC1 and NPC2 are essential for intracellular transport of free cholesterol. Affected individuals accumulate free cholesterol and glycosphingolipids in late endosomes and early lysosomes. So far, the pathomechanism of NPC is not fully understood.

Aim of our study was to examine the expression levels of genes engaged in cellular metabolic pathways in cell lines obtained of NPC patients and controls.

**Materials and Methods:** Total RNA was isolated from 10 NPC1 patients cell lines and 9 cell lines from control persons. Biotin-labeled cRNA samples were hybridized to HumanHT-12 v4.0 Expression Bead Chip. Row data obtained after microarray experiments were then analyzed with the Partek Genomic Suite v6.6 and Ingenuity Pathway Analysis program.

**Results:** Statistically significant alterations in expression were observed for three genes: *SOD1* coding for superoxide dismutase 1 (mean fold change, MFC, 2.5), *CTSK* coding for cathepsin K (MFC 2.5), and *CTSB* coding for ca-

thepsin B (MFC 1.5). We have found that in NPC cells the up-regulated genes were related to oxidative stress, autophagy, and apoptosis.

Conclusions: These preliminary results indicate that in humans activation of autophagy may enhance cell stress and eventually trigger the apoptotic pathway. This was already reported in NPC1 deficient mice as well as impaired proteolysis, which underlies autophagic dysfunction.

Our further work will include the validation of obtained data by qRT-PCR in human NPC fibroblasts.

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#### P06.59

#### Allelic variants associated with obesity and obesity related disorders in a Romanian cohort of overweight and obese elder males

R. Ursu<sup>1,2</sup>, P. Iordache<sup>1</sup>, N. Cucu<sup>3</sup>, C. Bohiltea<sup>1</sup>, V. Radoi<sup>1,2</sup>, C. Arsene<sup>2</sup>, G. Ursu<sup>4</sup>, I. Craciunescu<sup>4</sup>, G. Chelu<sup>2</sup>, R. Bohiltea<sup>1</sup>, C. Staicu<sup>5</sup>, V. Calota<sup>5</sup>, A. Voinoiu<sup>5</sup>, D. Mates<sup>4,5</sup>, A. Manolescu<sup>6</sup>, V. Jinga<sup>1,7</sup>;

<sup>1</sup>"Carol Davila" University of Medicine and Pharmacy, Department of Medical Genetics, Bucharest, Romania, <sup>2</sup>Synevo Romania, Bucharest, Romania, <sup>3</sup>University of Bucharest, Genetics Department of the Faculty of Biology, Bucharest, Romania, <sup>4</sup>"Agrippa Ionescu" Emergency Military Hospital, Clinic of Cardiovascular Disorders, Bucharest, Romania, <sup>5</sup>National Institute for Public Health, Bucharest, Romania, <sup>6</sup>University of Reykjavik, School of Science and Engineering, Reykjavik, Iceland, <sup>7</sup>Prof. Dr. Th. Burghele" Clinical Hospital, Department of Urology, Bucharest, Romania.

Background. Obesity is one of the world's pandemics. This research wishes to determine high-risk polymorphisms associated with obesity for the Romanian population. It is the first large obesity-related genome-wide association study in Romania.

Materials. Methods. A GWAS was performed on a Romanian cohort of 2024 males aged over 50 y.o. (408 obese, 894 overweight, 647 normalweight). The research included 700000 SNPs. Genotyping results were analyzed in correlation with obesity, obesity parameters (weight, BMI) and complications (diabetes, flebitis, hypertension, prostate cancer). Lifestyle risk-factors were considered (environment, smoking, alcohol, coffee). Allele frequencies were calculated and compared with literature.

Results. Intron 1 of the FTO gene (16q12.2) had the strongest signal (10-4 - 10-5 p values) when associated with weight, BMI and obesity level (18 highly correlated SNPs). A locus on chromosome 7 (7q31.1) showed lower associations (3 SNPs). FTO SNPs also revealed strong correlations with diabetes, especially in obese. Hypertension was found associated with polymorphisms on chromosomes 4, 12 and 13 (BANK1, PPP3CA, AT2B, FRY genes) but not obesity-mediated. Lifestyle risk-factors associated-SNPs also showed correlations with obesity, diabetes and hypertension. One variant in the FRY gene (13q13.1) yielded associations with both smoking and hypertension. Over 50 SNPs were correlated with prostate cancer, 28 revealing the strongest results (chromosomes 4,8,13,16,2,6).

Conclusions. The results revealed several gene clusters correlated with obesity and comorbidities. The implications of these variants for the Romanian population need to be further analyzed, replication studies having to be undertaken for confirmation.

The research is part of EU FP7 ProMark project.

#### P06.60

#### A 2.3 kbp deletion in the OTC gene resulting in distant splicing abnormality and lethal hyperammonemia

G. Storkanova<sup>1</sup>, J. Sokolova<sup>1</sup>, H. Vlaskova<sup>1</sup>, M. Hnizdova Bouckova<sup>1</sup>, L. Stolnaja<sup>1</sup>, H. Treslova<sup>1</sup>, L. Dvorakova<sup>1</sup>, O. Martincova<sup>1</sup>, J. Krijt<sup>1</sup>, M. Magner<sup>2</sup>, P. Jesina<sup>1</sup>, V. Kozich<sup>1</sup>;

<sup>1</sup>Institute of Inherited Metabolic Disorders, General University Hospital in Prague and First Faculty of Medicine, Charles University in Prague, Czech Republic, Prague 2, Czech Republic, <sup>2</sup>Department of Pediatrics and Adolescent Medicine, General University Hospital in Prague and First Faculty of Medicine, Charles University in Prague, Czech Republic, Prague 2, Czech Republic.

Introduction: X-linked ornithine carbamoyltransferase deficiency (OTCD) is the most common urea cycle defect. Human OTC gene is expressed almost exclusively in the liver. We present the results of molecular genetic analyses in a newborn male with typical biochemical markers of OTCD who died due to severe hyperammonemia and multiorgan failure seven days after birth.

Methods: Genomic DNA from blood and cDNA from postmortem liver were amplified by PCR, and respective PCR products were analyzed by Sanger sequencing. The extent of the genomic deletion was determined by sequencing PCR products containing the deletion boundaries, and confirmed by MLPA.

Results: Amplification of genomic DNA did not yield any PCR product of exon 2, Sanger sequencing did not reveal any other changes in the remaining exons. A 2,325 bp large deletion starting 2,113 bp upstream of exon 2 and ending 73 bp in intron 2 was identified in genomic DNA. Congruently,

deletion of exon 2 was also observed in liver mRNA (r:78\_216del, predicted p.Cys27Ilefs\*10). In addition, an unexpected splicing variant with deletion of exons 2-4 (r:78\_386del, p.Cys27\_Arg129del) was observed indicating possibly the presence of distant acting splicing regulatory elements in the 2,325 bp genomic DNA deletion.

Conclusions: This study demonstrates the important role of mRNA analysis for interpretation of genetic variants observed in genomic DNA and indicates that the OTC gene may carry splicing regulatory elements within the region spanning 2,325 bp between intron 1 and intron 2. Support: MH-CZ-DRO-VFM64165, PRVOUK-P24/LF1/3

#### P06.61

#### New mutations of the Twinkle mitochondrial helicase resulting in Perrault syndrome

B. A. Fekete, K. Pentelényi, A. Domokos, P. Balicza, A. Kékesi, A. Gál, M. J. Molnár; Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest, Hungary.

Perrault syndrome is a rare disease characterized by sensorineural hearing loss, ovarian dysfunction in females and neurological symptoms. The phenotypic heterogeneity is accompanied with genetic heterogeneity with the most commonly affected genes being HARS2, HSD17B4, LARS2 and CLPP. In one single report mutations in C10orf2 have been associated with the disease as well.

Hereby we present the case of a 20-year-old female with early onset progressive hypacusis and primary hypogonadism, which are the hallmarks of the Perrault syndrome phenotype. Additional clinical features are dysarthria, severe weakness in the limb muscles, truncal and limb ataxia, polyneuropathy and psychiatric symptoms. Serum lactate was elevated. MRI found: medulla oblongata and spinal cord atrophy. Genetic analysis of the HSD17B4 gene did not find pathogenic mutations. In her muscle tissue multiple mtDNA deletions were present. NGS analysis of the genes responsible for intergenomic communication detected compound heterozygous c.1196A>G and c.1358G>A mutations in the C10orf2 gene. The segregation analysis by Sanger sequencing found these mutations in her parents in heterozygous status.

Our observation confirms that Twinkle protein dysfunction is associated with Perrault syndrome. The severe neurological and psychiatric symptoms of our patient and the presence of medulla oblongata and spinal cord atrophy expand the phenotypic spectrum of the disorders caused by Twinkle mutations.

#### P06.62

#### therapeutic approaches in lysosomal storage diseases - identification of novel small molecule drugs in Fabry, Gaucher, Pompe and Niemann-Pick type C disease

J. Lukas<sup>1</sup>, S. Seemann<sup>1</sup>, A. Pockrandt<sup>1</sup>, C. Zheng<sup>1</sup>, M. Sharif<sup>2</sup>, A. Pews-Davtyan<sup>2</sup>, M. Beller<sup>2</sup>, A. Rolfs<sup>1,3</sup>;

<sup>1</sup>Albrecht-Kossel-Institute for Neuroregeneration, Rostock, Germany, <sup>2</sup>Leibniz Institute for Catalysis, Rostock, Germany, <sup>3</sup>Centogene AG, The Rare Disease Company, Rostock, Germany.

Lysosomal storage diseases (LSDs) form a group of rare, inherited, progressive disorders of lysosomal catabolism. The diseases are based on mutations in genes mainly encoding for soluble (or membrane-associated) lysosomal hydrolases as is the case for Fabry (FD), Gaucher (GD) and Pompe (PD) diseases. Integral membrane proteins and transporters can also be affected as in Niemann-Pick Type C (NPC) disease.

Several therapeutic interventions have been developed within the last two decades. Among the clinically approved therapies are namely bone marrow transplantation and enzyme replacement therapy. However, alternative treatment strategies need to be elaborated, because these conventional methods are limited by several means, e.g. non-responsivity of the central nervous system, the unavailability of ERT for certain diseases, etc.

We developed distinct cell culture models to test for candidate small molecule drugs for a clinical application in LSDs. Generally, the aim of our research was the identification and mechanistical examination of different compounds to act on a preferably broad range of distinct genotypes in LSDs and, consequently, attenuate disease course. Using a HEK293 cell-based system for the expression of mutant forms of  $\alpha$ -galactosidase A (FD) and  $\alpha$ -glucosidase (PD) enzymes we identified novel compounds and compound combinations from various structural and functional molecule classes being able to increase cellular activity of the mutant protein.

A compound derivatization and testing program has been initiated for known pharmacological chaperones in GD and NPC using patient fibroblast cells aiming at (1) optimising their activities and (2) getting insights into structure/function relationship of the compounds.

**P06.63****Effect of sepiapterin on three novel missense mutation forms of phenylalanine hydroxylase in hepatoma cells**

*M. Pecimonova<sup>1</sup>, A. Soltysova<sup>1,2,3</sup>, E. Polak<sup>4</sup>, M. Stojiljkovic<sup>4</sup>, Z. Levarska<sup>1</sup>, J. Krahulec<sup>1</sup>, L. Kadasi<sup>1,2,3</sup>;*

<sup>1</sup>Faculty of Natural Sciences, Department of Molecular Biology, Comenius University, Bratislava, Slovakia, <sup>2</sup>Institute for Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>3</sup>Institute of Virology, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>4</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia.

Phenylketonuria and its milder form, hyperphenylalaninemia, are metabolic, autosomal recessive genetic disorders, associated with the deficiency of enzymatic activity of enzyme phenylalanine hydroxylase (PAH). This enzyme catalyzes the essential conversion of L-phenylalanine to L-tyrosine in the presence of molecular oxygen, iron, and 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) which functions as a natural cofactor of PAH. BH4 has chaperone-like effect on PAH enzyme, which is functional only in tetrameric form and may also have a potential effect on PAH mRNA expression in hepatoma cells.

Until now, more than 950 variants were identified in the PAH gene. Novel missense mutations p.F233I, p.R270I and p.F331S in this gene were found in the Slovak population. Previously, we performed PAH functional assays for testing the impact of these mutations on the functionality of PAH protein using prokaryotic expression system.

In this work, we focused on the expression of PAH mutated proteins in eukaryotic cell line HepG2 in the presence of sepiapterin which acts as the precursor of BH4. However some authors report that BH4 has no effect on PAH mRNA expression and has only effect on proper protein folding, other research papers report that PAH mRNA expression is increased in presence of BH4. Therefore we performed Real Time PCR and Western blot analyses to investigate the impact of sepiapterin on the level of mRNA and mutated PAH proteins.

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**P06.64****Phenylketonuria, clinical and molecular aspects in a group of Romanian patients**

*R. C. Vultură<sup>1</sup>, M. Mager<sup>2</sup>;*

<sup>1</sup>Univ. of Medicine and Pharmacy, Dept. of Cell and Molecular Biology, Cluj-Napoca, Romania, <sup>2</sup>Univ. of Medicine and Pharmacy, Dept. of Neurology, Cluj-Napoca, Romania.

Phenylketonuria is an autosomal recessive disease that, if is untreated, will impaire cognitive development resulting from a neurotoxic effect of hyper-phenylalaninemia (HPA); the associated clinical phenotype varies and the potential genotype-phenotype correlations would help in understanding the pathophysiology of this disease. Its metabolic phenotype is accountable to multifactorial origins both in nurture [where the normal nutrition introduces L-phenylalanine] and in nature, where mutations (>520 alleles) occur in the PAH gene. The allelic variation at the PAH locus yield greater or lesser risk of impaired cognitive development according to the degree of HPA. The clinical evaluation of a group of 20 PKU patients (with different stage of treatment) from neurology section of Cluj Children Hospital, Romania, was done according to The München development scale for the children with mental age below 3 years, with The Simon-Binet test for children with mental age 3-7 years and with The Raven's Progressive Matrices for those above 7 years. Biochemical phenotypes associated were included in severe PKU when the level of Phe is above 1200 µmol/l, moderate PKU (Phe: 600-1200 µmol/l) or mild HPA for Phe: 120-600 µmol/l. The leukocyte DNA was isolated and using polymerase chain reaction with subsequent restriction analysis, we identified patients homozygous for mutation R408W (more than 50%), patients compound heterozygotes for R408W/L48S, R408W/R413P, R261Q/IVS12+1>a, Y268C/ IVS12+1>a, or R408W/R261Q mutations. Our data provide that the highest degree of concordance genotype-phenotype was found in patients with null/null genotypes and evidence that a simple genotype-phenotype correlation does exist in this group of patients.

**P06.65****Plasma and tissue miRNA signatures to understand the pathophysiology in propionic acidemia**

*A. Rivera-Barahona<sup>1</sup>, A. Fulgencio-Covián<sup>1</sup>, B. Pérez<sup>1,2</sup>, C. Pérez-Cerdá<sup>2</sup>, M. Ugarte<sup>2</sup>, E. Richard<sup>1,2</sup>, L. R. Desviat<sup>1,2</sup>;*

<sup>1</sup>Centro de Biología Molecular, Madrid, Spain, <sup>2</sup>Centro de Diagnóstico de Enfermedades Moleculares, Madrid, Spain.

miRNAs are regulatory short non-coding RNAs involved in many cellular processes and pathological conditions, thus they have emerged as potential biomarkers and therapeutic targets in human diseases. In this work we have

analysed the miRNA profile in plasma samples from patients with the rare metabolic disorder propionic acidemia (PA) and in different tissues from the hypomorphic murine model. PA is characterized by the toxic intracellular accumulation of propionyl-CoA due to propionyl-CoA carboxylase (PCC) enzyme deficiency and usually presents as a neonatal form with patients developing neurological deficits and cardiomyopathy in the long-term. We have used a qRT-PCR miRNome panel to analyse the expression of 752 miRNAs in *Pcca<sup>-/-</sup>* (A138T) mouse liver and in plasma samples from PA patients and controls. In mouse, we have found 14 significantly dysregulated miRNAs from which three also exhibited altered plasma levels in PA patients. In mouse tissues we selected miR-34a-5p, miR-338-3p and miR-350 to investigate their potential targets in relation to PA pathology. The levels of the three miRNAs were found increased in brain and heart of the hypomorphic mice at different ages, correlating in some cases with a decrease in specific predicted targets such as BCL2, MEK1, p38, JNK and ATP5G1, involved in apoptosis, stress-signalling and mitochondrial function. Taken together, our results point out to the role of miRNAs in PA pathophysiology and underscore their potential use as biomarkers of the disease.

**P06.66****New insights into renal amino acid reabsorption**

*C. Vilches<sup>1</sup>, M. López de Heredia<sup>1</sup>, S. Bodoy<sup>2</sup>, M. Espino<sup>1,2</sup>, E. Boiadjieva<sup>3</sup>, E. Prat<sup>1,4</sup>, L. Gómez<sup>1</sup>, R. Artuch<sup>5</sup>, F. Verrey<sup>2,6</sup>, M. Palacín<sup>2,6</sup>, V. Nunes<sup>2,6</sup>, \*authors sharing leadership;*

<sup>1</sup>Human Molecular Genetics Laboratory, IDIBELL, U730 CIBERER, L'Hospitalet de Llobregat, Barcelona, Spain, <sup>2</sup>Institute for Research in Biomedicine (IRB Barcelona), U731 CIBERER, Barcelona, Spain, <sup>3</sup>Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland, <sup>4</sup>Genetics Unit, Physiological Sciences II Department, Medicine Faculty, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain, <sup>5</sup>Clinical Biochemistry Department and Institute of Research Pediatrics, U703 CIBERER, Barcelona, Spain, <sup>6</sup>Biochemistry and Molecular Biology Department, Biology Faculty, University of Barcelona, Barcelona, Spain.

Introduction: The generation of mouse models for heteromeric amino acid transporters has been proved to be a powerful tool to understand the renal amino acid transport in mammals and subsequently our knowledge about aminoacidurias.

Materials and Methods: We have generated and characterized a double-loss-of-function mouse model for the two basolateral amino acid transporters LAT2 and TAT1 (hereafter, dKO). Animals were treated with a high protein content diet, amino acids were quantified in urine and plasma samples to assess renal function, and expression of amino acid transporters was analyzed by RT-qPCR in the dKO, the single KOs and control mice.

Results: We found a decreased reabsorption of aromatic and neutral amino acids and to a lesser extent, of basic ones and proline, which is exacerbated under rich protein diet. A strong renal phenotype in the dKO versus single ones supports a coordinated function of LAT2/4F2hc and TAT1 on the basolateral transport in renal reabsorption. The expression analyses demonstrate the upregulation of one particular isoform of the basolateral transporter y+LA T1, and in a lesser extend of SNAT3.

Conclusion: There is cooperation between LAT2/4F2hc and TAT1 in the renal reabsorption, where TAT1 supplies neutral amino acids for LAT2 exchanger. The dKO model presents a significant remaining tubular reabsorption of neutral amino acids suggesting the existence of compensation by other basolateral transporters. y+LAT1 and SNAT3 seem to be two of them. Altogether, our results provide new insights into the in vivo mechanisms of amino acid resorption in the kidney.

**P06.67****Use of shRNAs on patients' cells as a long-term substrate reduction therapy approach for Sanfilippo C disease**

*N. Benetó<sup>1</sup>, I. Canals<sup>1,2</sup>, L. Vilageliu<sup>1</sup>, D. Grinberg<sup>1</sup>;*

<sup>1</sup>Dept. Genetics, Fac. Biology, University of Barcelona, CIBERER, IBUB, Barcelona, Spain,

<sup>2</sup>Stem Cells, Aging and Neurodegeneration Group, Lund Stem Cell Center, University Hospital, Lund, Sweden.

Sanfilippo C syndrome is a rare lysosomal storage disorder caused by mutations in the HGSNAT gene, which encodes an enzyme involved in heparan sulphate (HS) degradation. The enzyme deficiency causes the storage of partially degraded HS molecules inside the lysosome. The disease has an autosomal recessive inheritance pattern and is characterized by a severe and progressive neurodegeneration for which no effective treatment exists. Previously, we demonstrated, on Sanfilippo C patients' fibroblasts, that the use of siRNAs targeting EXTL2 and EXTL3, genes involved in HS synthesis, could be effective as a short-term substrate reduction therapy (SRT). Here, we have used five different lentiviruses encoding shRNAs targeting EXTL2, to analyse the effect of a long-term treatment. All the shRNAs caused

a notable reduction in the mRNA levels (around 90%) of the EXT2 gene sixty days post-infection. Moreover, immunocytochemistry analyses showed a clear decrease of the HS amounts after treatment.

Due to the good results obtained on patients' fibroblasts, now we are using the most effective shRNAs on induced pluripotent stem cells (iPS cells) derived from patients' fibroblasts. We have set up the conditions for the differentiation of those iPS cells to neurons, the most affected cell type in this disease, and we are going to perform the same assays on them.

Our results confirm the usefulness of shRNAs as a long-term SRT, becoming a promising approach for a future therapeutic option for Sanfilippo C syndrome.

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#### P06.68

#### Identification of rare genetic variants in patients with non-syndromic early-onset obesity using a pooled DNA sequencing approach

F. Bou<sup>1</sup>, C. Serra-Juhé<sup>1,2,3</sup>, G. Martos-Moreno<sup>4,5,6</sup>, B. Rodríguez-Santiago<sup>7</sup>, R. Flores<sup>1,2,3</sup>, J.

Argente<sup>4,5,6</sup>, L. Pérez-Jurado<sup>1,2,3</sup>;

<sup>1</sup>Genetics Unit, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain, <sup>4</sup>Departments of Pediatrics & Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, Madrid, Spain, <sup>5</sup>La Princesa Research Institute, Madrid, Spain, <sup>6</sup>Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain, <sup>7</sup>Quantitative Genomic Medicine Laboratories (qGenomics), Barcelona, Spain.

Studies trying to elucidate the pathophysiology of obesity consistently describe a highly heterogeneous disorder at the clinical and molecular level, with high heritability. At least 10 genes, mostly with recessive inheritance, have been reported to cause monogenic severe obesity, and there are a few more candidates of strong effect in association studies.

The aim of this study was to establish the contribution of rare genetic variants in candidate genes to early-onset obesity (BMI>3SDS, <3y). Using a pooled DNA sequencing approach, which allows analyzing many samples with reduced costs, we screened 15 candidate genes for obesity in a cohort of 480 patients and 480 controls (BMI<P50). We focused on very rare variants found in single or few individuals per cohort.

Seven of the 15 genes (*BDNF*, *FTO*, *MC3R*, *MC4R*, *NEGR1*, *PPARG* and *SIM1*) were differentially represented between patients and controls; we identified 30 rare variants in patients and 5 in controls ( $p=0.0001$ ). The difference of probably pathogenic variants (15 in patients vs 1 in controls) was also significant ( $p=0.0005$ ); all were single allele changes and none of the individuals carried more than one variant.

Our data reveal a higher burden of rare and probably pathogenic heterozygous variants in several candidate genes in patients with severe early-onset obesity compared to controls. Our results reinforce the role of the melanocortin pathway and bring to light other genes that may carry highly penetrant obesogenic single allele variants, like *FTO*, *PPARG* and *BDNF*.

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#### P06.69

#### Rare copy number variants reveal novel genes and pathways involved in early-onset obesity

C. Serra-Juhé<sup>1,2,3</sup>, G. Martos-Moreno<sup>4,5,6</sup>, F. Bou<sup>1</sup>, B. Rodríguez-Santiago<sup>7</sup>, R. Flores<sup>1,2,3</sup>, J. González<sup>8,9</sup>, J. Argente<sup>4,5,6</sup>, L. Pérez-Jurado<sup>1,2,3</sup>;

<sup>1</sup>Genetics Unit, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain, <sup>4</sup>Departments of Pediatrics & Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús, Universidad Autónoma de Madrid, Madrid, Spain, <sup>5</sup>Hospital de la Princesa Research Institute, Madrid, Spain, <sup>6</sup>Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain, <sup>7</sup>Quantitative Genomic Medicine Laboratories (qGenomics), Barcelona, Spain, <sup>8</sup>Centro de Recerca en Epidemiología Ambiental (CREAL-ISGlobal), Barcelona, Spain, <sup>9</sup>Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain.

Introduction: Obesity is a multifactorial disorder with high heritability (50-75%), probably higher in early-onset and severe cases. Although rare monogenic forms and several genes and regions of susceptibility, including CNVs, have been defined, the genetic causes underlying the disease still remain largely unknown.

Methods: We studied a cohort of Spanish children with severe (BMI>3SDS) non-syndromic early-onset (<3y) obesity (EOO). We obtained molecular karyotypes of 157 cases. Large (>100kb) and rare (<1/2,000 controls) CNVs were validated, segregated in the family, and studied in a larger sample (323

EOO cases/480 controls). Additionally, mutation analysis of the genes altered by CNVs (n=16) was completed in both cohorts by NGS using a pooled DNA strategy.

Results: A higher burden of duplication-type CNVs was detected in EOO cases versus controls (OR=1.85, p-value=0.008). Likely pathogenic CNVs included duplications of glutamate receptor (GRIK1, GRM7), the X-linked gastrin-peptide receptor (GRPR) and the NPY genes, all inherited from obese parents. Results were replicated by MLPA in the extended cohort. By NGS focusing the analysis on rare variants, we identified a missense mutation in NPY, a nonsense mutation in GRIK1, and 5 missense mutations in GRPR in EOO cases, but no mutations in controls.

Conclusions: Our data reveal a higher burden of rare CNVs and point mutations in patients with EOO compared to controls. Genes altered by CNVs are candidates for contributing to the pathogenesis of morbid EOO. Among these, NPY, GRPR and two glutamate receptors are likely involved in highly penetrant, monogenic and familial obesity.

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#### P06.70

#### Vitamin E treatment in Smith-Lemli-Opitz syndrome patients - experiences of follow up

K. Koczak<sup>1</sup>, L. Horváth<sup>2</sup>, G. P. Szabó<sup>3</sup>, Z. Korade<sup>4</sup>, N. A. Porter<sup>5</sup>, I. Balogh<sup>1</sup>;

<sup>1</sup>Department of Laboratory Medicine, Division of Clinical Genetics, University of Debrecen, Debrecen, Hungary, <sup>2</sup>Department of Pharmaceutical Surveillance and Economy, University of Debrecen, Debrecen, Hungary, <sup>3</sup>Department of Pediatrics, University of Debrecen, Debrecen, Hungary, <sup>4</sup>Department of Psychiatry, Vanderbilt University, Nashville, TN, United States, <sup>5</sup>Department of Chemistry, Vanderbilt University, Nashville, TN, United States.

Introduction: Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation syndrome caused by 7-dehydrocholesterol reductase enzyme deficiency. Decreased cholesterol, elevated 7-dehydrocholesterol (7-DHC) and oxidative derivatives of 7-DHC are putative pathophysiological factors of SLOS. According to literature vitamin E is effective in reducing 7-DHC-derived oxysterols in cultured SLOS fibroblasts. We have started a study on clinical effects of vitamin E in SLOS in 2014.

Materials and Methods: Seven SLOS patients were enrolled in the study receiving 230 mg (age: 4-10 years) or 2x230 mg (above 10 years) RRR-alpha-tocopherol acetate beside cholesterol supplementation. Plasma vitamin A and E concentrations were monitored regularly by HPLC. Behavioral effects of the therapy were determined using the Aberrant Behavior Checklist (ABC) at baseline, after 6 and 12 months. During follow-up, upon anecdotal reports of the parents, we created another questionnaire focusing on sleeping habits and photosensitivity as well in addition to behavior.

Results: Patients were vitamin E deficient prior to therapy, while vitamin A concentrations were normal in all but one patient. Absorption of the vitamin was satisfactory, plasma vitamin E concentrations showed normalization in all patients. According to ABC improvement in irritability and stereotypic behavior was detected in one patient. Using our questionnaire positive effects on self-injurious and stereotypic behavior, improvement of sleeping habits and photosensitivity were observed in four patients.

Conclusions: Vitamin E deficiency is likely to be the consequence of higher utilization and not inadequate absorption and can be normalized using high treatment doses. Our results suggest potential benefit of vitamin E treatment in SLOS.

#### P06.71

#### Sandhoff disease : a case report with new mutation

S. Seyhan, H. Önder Yilmaz, A. Cansu, A. H. Çebi, M. Ikbal;

Karadeniz Technical University Medicine Faculty, Trabzon, Turkey.

Sphingolipidoses are inherited lysosomal storage diseases which are result of the accumulation of sphingolipids therefore progressive neurodegenerative findings revealed. In Sandhoff disease there are lack of activities of total hexosaminidase (A and B components) with beta subunit gene mutation and it lead to the accumulation of GM2 gangliosides in neural tissue. It is divided into three types acute (infantile), juvenile (late infantile) and adult (chronic). The incidence of sphingolipidoses in Turkish Society is 4,1/100,000 in live birth. In Sandhoff disease the accumulation of sphingolipids in macular retinal ganglion cell causes typical „Japanese flag“ formation of the image.

In this study, we present a 3-years-old male who was brought to our pediatric neurology clinic because of fatigue, slow movement, inactivity since his birth. On physical examination we determine decreased deep tendon reflexes, mild coarse facies and cherry spot on ocular fundus. Delays in all developmental stages were detected in Denver-2 test. Parents are relative. Due to diagnosis of Lysosomal enzyme levels in white blood plasma cells

hexosaminidase A and B levels were determined as 20% lower limit we made HEXB gene whole exon sequence analysis. As a result we determine the patient p.S210P(c.628T>C)(homozygous). The analysis of known mutations that are sent from the patient's parent were determined as p.S210P(c.628T>C)(heterozygous). We present this case because it is a new mutation in the HEXB gene.

#### P06.72

#### Characterization of HEK293 cell lines stably expressing mutated *SRD5A3* variants to mimick *SRD5A3-CDG*

A. Buczkowska<sup>1</sup>, P. Link-Lenczowski<sup>2</sup>, L. Borowski<sup>1</sup>, A. Fogtman<sup>1</sup>, M. Koblowska<sup>1</sup>, D. J. Lefebvre<sup>3</sup>, A. Dziembowski<sup>1</sup>, E. Swiezewska<sup>1</sup>;

<sup>1</sup>Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland,

<sup>2</sup>Jagiellonian University Medical College, Krakow, Poland, <sup>3</sup>Department of Neurology, Translational Metabolic Laboratory, Radboud University Medical center, Nijmegen, Netherlands.

Attachment of carbohydrate chains to proteins is a common post-translational modification occurring ubiquitously in mammalian cells. N-Glycosylation pathways use the lipid carrier dolichol (Dol) as an obligate precursor. Mutations in the genes encoding enzymes involved in the synthesis of Dol cause deficiencies in cellular function and lead to a variety of debilitating diseases called congenital disorders of glycosylation type I. One of the variants, *SRD5A3-CDG*, is caused by nonsense mutations in the gene encoding polyprenol reductase, *SRD5A3*, which plays a crucial role in the dolichol cycle by catalyzing conversion of polyprenol to dolichol. Decreased content of dolichyl phosphate affects the assembly of the Dol linked Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> glycan and its transfer to proteins upon N-glycosylation.

Flp-In technology was used to obtain several HEK293T cell lines expressing miRNA silencing endogenous *SRD5A3* and simultaneously complemented with mutated variants of *SRD5A3*. Biochemical analysis of model cell lines demonstrated that expression of mutated *SRD5A3* led to aberrations in lipid metabolism and in protein glycosylation. Additionally, using microarray analysis, we investigated the differences in gene expression profiles between HEK293 cell lines expressing mutated *SRD5A3* and control. This analysis revealed more than 100 genes with modulated expression (up/down-regulated) compared to the mock transfected cells. Functional identification of these genes is in progress.

Results of these analyses will help to understand the variability of the clinical symptoms observed for *SRD5A3-CDG* patients.

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#### P06.74

#### Molecular diagnosis of Wilson disease by Next Generation Sequencing

V. Parrillas, E. Sevilla, C. Rodríguez, C. Camarena, E. Vallespín, A. Carazo, V. Montaño, R. Mena, J. Silla, P. Lapunzina, S. Rodríguez Novoa;  
Hospital Universitario La Paz, Madrid, Spain.

Introduction: Wilson's disease (WD, OMIM #277900) is an autosomal recessive disorder of copper metabolism that produces intracellular copper accumulation in liver, brain, kidney and cornea and may lead to hepatic cirrhosis and neurological damage. WD affects from 1/30000 to 1/100000 individuals worldwide. *ATP7B* gene (MIM #606882), responsible for WD, is a copper transporter P-ATPase that plays a key role in incorporating copper into ceruloplasmin and moving excess copper out of the liver. Over 200 different mutations have been identified in the *ATP7B* gene.

Materials and Methods: Patients with clinical evidence of Wilson's disease were remitted to our service for genetic testing. Analysis of the *ATP7B* was

done by Next Generation Sequencing (NGS). Multiplex Ligation-dependent Probe Amplification (MLPA) was performed in all patients in order to detect deletions/duplications in the *ATP7B* gene.

Results: Twenty unrelated patients were analyzed. Four of them carried pathogenic variants at *ATP7B*. Table shows their biochemical characteristics and genotypes. Three patients were simple heterozygous for known mutations in *ATP7B*. One patient was compound heterozygous.

Conclusions: Most of the patients with mutations in the *ATP7B* gene are simple heterozygous. This data can suggest the importance of conducting a thorough analysis of gene promoter *ATP7B* as well as finding new targets for molecular diagnosis of EW.

#### P06.75

#### Diagnostic tests for hereditary xanthinuria

I. Sebesta<sup>1,2</sup>, B. Stiburkova<sup>2,3</sup>, J. Krijt<sup>4</sup>;

<sup>1</sup>Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University, Prague, Czech Republic, <sup>2</sup>Institute of Inherited Metabolic Disorders, Prague, Czech Republic, <sup>3</sup>Institute of Rheumatology, Prague, Czech Republic,

<sup>4</sup>Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Introduction: Hereditary xanthinuria is an autosomal recessive inherited metabolic disorder due to deficiency of the xanthine dehydrogenase/ oxidase (XDH/XO - type I), and is characterized by low concentration of uric acid (UA) in blood and urine and high concentration of urinary xanthine. Type II results from a combined deficiency of XDH/XO and aldehyde oxidase. Patients present with hematuria, renal colic, urolithiasis or even acute renal failure. Molybdenum cofactor deficiency (type III) is characterized by the lack of sulfite oxidase as well as XDH/XO and aldehyde oxidase activities. About 150 cases have been described so far.

Materials and Methods: Hypouricemic patients were found from 3 800 blood and urine samples. Following tests were set up: a) evaluation of UA in serum and urine with exclusion of secondary causes of hypouricemia b) estimation of urinary xanthine, c) allopurinol loading test and finally XDH/XO activity assay in plasma with molecular genetic analysis.

Results: Nine Czech cases were detected, which is one of the largest group worldwide, in the terms of the number of patients. All individuals had profound hypouricemia as the first sign. Urinary concentrations of xanthine were in the range of 170-598 mmol/mol creatinine (ref.range: < 30 mmol/mol creatinine). XDH/XO activities in plasma in two cases were 0 and 0.37 pmol/h/mL of plasma (ref.range: 3.2-9.2 pmol/h/mL of plasma). The nonsense heterozygous mutation p.R825X was found in two patients.

Conclusions: Hereditary xanthinuria is probably not so rare as previously thought. Patients with unexplained hypouricemia need detailed purine metabolic investigation.

#### P06.76

#### Lethal Zellweger spectrum syndrome due to a novel mutation in the *PEX3* gene

E. M. Honey<sup>1</sup>, M. Dercksen<sup>2</sup>, L. J. Mienie<sup>2</sup>, B. C. Vorster<sup>2</sup>;

<sup>1</sup>University of Pretoria, Pretoria, South Africa, <sup>2</sup>North-West University, Potchefstroom, South Africa.

Background: Zellweger spectrum syndromes (ZSSs) are a heterogeneous group of autosomal recessive neurodegenerative disorders that affect multiple organ systems. A high incidence of ZSSs in the South African population has been observed in the last 20 years. Most of these patients were diagnosed in the first 8-10 weeks after birth and lived an average of 2 years. Three cases however presented with a severe phenotype and these patients died soon after birth.

Patient: A neonate, presenting with facial and finger dysmorphia as well as

Probands	Genotype of <i>ATP7B</i> (NM_000053.3.4)					Biochemical parameters		
	DNA Sequence	Protein Sequence	Type	Exon Localization	Intron Localization	Serum Copper (mg/dL)	Urine Copper (mg/24h)	Serum Ceruloplasmin (mg/dL)
1	Father: c.122 A>G Mother: c.3060+5G>T Brother 1: c.122 A>G c.3060+5G>T Brother 2: c.122 A>G c.3060+5G>T	c.122 A>G c.3060+5G>T	p.N41S	Missense Splicing	2	13 29	No data	9.23
2	c.879G>C	c.879G>C	p.E293D	Missense	2		No data	No data
3	c.1934T>G	c.1934T>G	p.M645R	Missense	6		77 Undetectable	17.7
4	c.1934T>G	c.1934T>G	p.M645R	Missense	6		14 44	4.6

lactic acidosis after birth, was recently referred to the Potchefstroom laboratory for inborn errors of metabolism, South Africa for a metabolic work-up. The patient unfortunately died a day after birth.

**Results:** Initial metabolic investigations on plasma indicated an elevated level of C26 as well as C24/C22 and C26/C22 ratios. In addition, increased pipecolic acid was also observed. A ZSS was suspected after plasma bile acids analysis revealed elevated dihydroxycholestanic acid, trihydroxycholestanic acid and C29-dicarboxylic bile acid. Complementation studies, for accessing peroxisomal biogenesis disorders on fibroblasts, were done and revealed PEX3 gene as a probable candidate. The sequenced mutation analysis of the PEX3 gene showed that the patient had a novel homozygote frameshift mutation namely c.203\_204dup (p.Val69Glnfs\*9).

**Conclusions:** It was concluded that this pathogenic mutation results in the expression of a non-functional truncated protein and consequently a severe and fatal phenotype. Our findings on and experience in ZSSs gives merit to the investigation of peroxisomal biogenesis disorders in neonates presenting with dysmorphia and metabolic decompensation.

## P07 Immunology and hematopoietic system

### P07.01

#### Somatic mutation in the HLA genes in a patient with acute myeloid leukemia (AML) prior to hematopoietic stem cell transplantation (HSCT): a case report

V. V. Zakharova, O. A. Shragina, M. A. Maschan, E. V. Orehova, M. A. Kournikova, E. V. Raikina;  
Dmitry Rogachev Federal Research Centre of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.

**Introduction:** The most important factor that can influence HSCT outcome is HLA matching. Recent publications have pointed somatic mutations resulting in copy-neutral loss of heterozygosity (CN-LOH) for part or full HLA haplotype.

**Patient:** A 15-year old female previously diagnosed with AML-M5 was referred to clinic with leukemia relapse. Bone marrow showed 97% of blasts. Cytogenetic analysis detected CALM-AF10 fusion gene. FLT3, NPM1 mutations were negative. Unrelated donor search was initiated. But, after two blocks of re-induction chemotherapy she didn't achieve remission and developed sepsis, pneumonia. So, haploidentical HSCT was a treatment of choice. Unfortunately, the evidence of disease progression made it impossible. Patient had to choose palliative care.

**Result:** HLA-typing by SBT and NGS indicated that the patient was homozygous: A\*02:01-B\*18:01-Cw\*07:01-DRB1\*11:04-DQB1\*03:01. Confirming result was obtained in registry of unrelated stem cell donors. Mother's typing by SBT: A\*03:01,26:01-B\*35:03,56:01,-Cw\*12:03,01:02-DRB1\*11:01,07:01-DQB1\*03:01,03:03. There was no common haplotype between mother and daughter. The maternity was confirmed by STR. Testing of patient by SSP revealed a second haplotype (Bw6): A\*03:01-B\*35:03-Cw\*12:03-DRB1\*11:01-DQB1\*03:01.

**Conclusion:** We found HLA-diploid normal cells in minority and malignant cells with acquired uniparental disomy. SSP unlike sequencing doesn't depend on balanced PCR amplification of the two alleles present at a locus and might detect the two haplotypes. Bw6 loss confirms the hypothesis from previous cases where Bw6 alleles have been found to the target of HLA malignant eradication. This case shows that LOH may lead to erroneous typing results. Homozygotes should be confirmed by SSP, typing of buccal swabs, blood samples in remission or creating a family tree by typing the parents.

### P07.02

#### Unique concurrence of monosomy of chromosome 7 and KMT2A gene rearrangement in a patient with AML

Z. Litwinska<sup>1</sup>, A. Pietrzylk<sup>2</sup>, E. Studniak<sup>2</sup>, R. Guzicka-Kazimierczak<sup>3</sup>, B. Zdziarska<sup>3</sup>;  
<sup>1</sup>Department of General Pathology, Pomeranian Medical University, Szczecin, Poland,  
<sup>2</sup>Cytogenetic Unit, Pathology Department, Pomeranian Medical University, Szczecin, Poland, <sup>3</sup>Department of Hematology, Pomeranian Medical University, Szczecin, Poland.

Acute myeloid leukemia (AML) is a type of myeloid lineage malignancy, heterogeneous in terms of morphology, immunophenotype as well as cytogenetic, molecular genetics and clinical features. Chromosomal rearrangements, detected in classical cytogenetic analyses and FISH have a significant impact on detecting and classifying type of AML as well as choice of treatment and determining prognosis. Both monosomy of chromosome 7 and KMT2A gene rearrangements are aberrations of remarkable prognostic value.

We present a case of 38-year-old male patient referred to Hematology Department with suspicion of AML. The bone marrow sample was aspirated with white blood cell count measuring 30x10<sup>3</sup>/µL. Classical cytogenetic analysis was performed according to the standard protocol. Patient's karyotype was

determined as: 45,XY,-7,t(9;11)(p21;q23)[19/20]/ 46,XY[1/20]. 90% of evaluated metaphases presented monosomy of chromosome 7 and translocation t(9;11)(p21;q23) resulting in fusion gene KMT2A-MLLT3. Both aberrations were confirmed by FISH.

Monosomy of chromosome 7 occurring with translocation t(9;11)(p21;q23) is extremely rare in AML, with only few cases ever described. As both cell clones were assessed to be in a similar percentage, it was difficult to decide which one of them was the primary abnormality. It is problematic to predict the course of disease in our patient as monosomy of chromosome 7 is linked with adverse prognosis and this specific KMT2A-MLLT3 fusion gene is a factor of moderate prognosis, contrary to majority of KMT2A rearrangements, correlated with poor outcome.

The concurrence of these two aberrations, differently affecting prognosis, poses a diagnostic and therapeutic challenge and requires further clinical and cytogenetic follow-up.

### P07.03

#### A comprehensive next generation sequencing gene panel focused on unexplained anemia

F. E. Smith<sup>1</sup>, D. Brawand<sup>1</sup>, L. Steedman<sup>1</sup>, M. W. Oakley<sup>1</sup>, C. Wall<sup>1</sup>, P. Rushton<sup>1</sup>, M.

Allchurch<sup>2</sup>, K. Sibson<sup>3</sup>, C. Hemmaway<sup>2</sup>, D. Rees<sup>4</sup>, S. Thein<sup>5</sup>, B. Clark<sup>1</sup>;

<sup>1</sup>Viapath at King's College Hospital, London, United Kingdom, <sup>2</sup>Haematology, Queen's Hospital, Romford, United Kingdom, <sup>3</sup>Haematology Department, Great Ormond Street Hospital, London, United Kingdom, <sup>4</sup>King's College Hospital, London, United Kingdom,

<sup>5</sup>National Heart, Lung and Blood Institute/NIH, Bethesda, MD, United States.

Congenital anemia is difficult to diagnose once common causes have been excluded; for example 80% cases of congenital non-spherocytic hemolytic anemia are undiagnosed once pyruvate kinase and G6PD deficiencies have been excluded using phenotypic analysis. We describe a next generation sequencing strategy, targeting 147 genes, to facilitate the diagnosis of these conditions. The coding regions, splice sites and 200 bp into the untranslated regions were examined in each gene. All clinically significant variants were confirmed by Sanger sequencing, including confirmation in any appropriate family members.

Illumina MiSeq data was analysed using a bespoke bioinformatics pipeline, which has been validated to a UK certified standard. The pipeline implements detection of genetic variants using multiple base callers and discovery of copy number variants based on sequencing depth. Variants are annotated with information from ClinVar, and population frequency data (ExAC and 1000 genomes project). All genes are sequenced in every individual but data analysis can easily be restricted to virtual subpanels, excluding analysis of genes not requested. We present three cases; one hereditary pyropoikilocytosis, one congenital dyserythropoietic anaemia and one haemoglobinopathy due to a beta globin locus control region deletion; highlighting the diagnostic utility of the panel as well as the underlying bioinformatics analysis. Identifying pathogenic variants in unexplained anaemia cases is important as it facilitates prognosis and treatment, and allows prenatal diagnosis to be offered in future. To date the panel has assessed 35 cases of anemia with unknown cause and has made a definitive diagnosis in 29 (83%).

### P07.04

#### MVK, NLRP3, TNFRSF1A and MEV gene mutation profile of patients with autoinflammatory diseases: report of 286 pediatric patients from Northern Anatolia

E. Altundag<sup>1</sup>, G. Ogur<sup>1</sup>, U. Abur<sup>1</sup>, H. Mutlu Albayrak<sup>1</sup>, M. Celiksoy<sup>2</sup>, A. Paul<sup>1</sup>, M. Karadag<sup>1</sup>, A. Yildiran<sup>2</sup>;

<sup>1</sup>Ondokuz Mayis University Medical Faculty Department of Genetics, Samsun, Turkey,

<sup>2</sup>Ondokuz Mayis University Medical Faculty Department Of Pediatric Allergy And Immunology, Samsun, Turkey.

**INTRODUCTION:** The spectrum of MEV, TNFRSF1A, MVK and NLRP3 gene mutations of autoinflammatory diseases in different ethnical groups is yet not fully studied. Here we present results from 286 pediatric patients from Northern Turkey.

**MATERIAL-METHOD:** MEV (exons 2,3,5,10), TNFRSF1A (exon 2-7), MVK (exon 2-11) and NLRP3 (exon 3) were sequenced in 286 pediatric patients. Two groups were studied: one included 194 patients with AID and the other, 92 FMF patients without MEV gene mutations (FMF-WT).

**RESULTS:** In AID group, 10% NLRP3, 6% TNFRSF1A, 21% MVK and 32% MEV mutations were detected. Overall mutation rate was %38; the rate was 3% for pathological mutations.

In FMF-WT group, 20% NLRP3 and 29% MVK mutations were detected. When Tell-Hashomer criteria was included, 36% patients yielded NLRP3 mutations (no mutation for TNFRSF1A). Overall mutation rate was %41 and pathological mutation, was %3.

MEV sequencing in AID yielded mutations in %32 patients (34% M694V, 17% E148Q, 13% V726A, 9% M680I, 7% P369S, 20% others).

Briefly, 118 out of 286 patients (41%) yielded mutations including low pe-

netrance variants. 9 (3%) showed pathological mutations (five V377I heterozygous, one heterozygous N205S c.614A>G novel mutation for MVK; two I313V mutations for NLRP3 and one N116S mutation for TNFRSF1A).

**DISCUSSION:** This is the first study relevant to MEFV, NLRP3, MVK and TNFRSF1A gene mutation profile in pediatric AID from Turkey. The results are similar to studies in literature in terms of distribution of all variants. However lower rates were observed for pathological mutations. Mutation spectrum was also limited in our study.

## P07.05

### Effects of gliadin and genetic background in tight junction structure integrity

*A. Jauregi-Miguel<sup>1</sup>, I. Santin<sup>2</sup>, K. Garcia-Etxebarria<sup>1</sup>, I. Romero-Garmendia<sup>1</sup>, L. Plaza-Izurieta<sup>1</sup>, J. Bilbao<sup>1</sup>;*

<sup>1</sup>University of Basque Country, Leioa, Spain, <sup>2</sup>BioCruces Research Institute, Barakaldo, Spain.

Tight junction structures (TJ) are crucial for intestinal epithelium homeostasis as they control the paracellular flux and maintain the apico-basal polarity of intestinal cells. In celiac disease (CD), disrupted barrier function and increased paracellular permeability are observed, and these could be due to TJ disassembly caused by gliadin-induced innate and adaptive immune responses. Gene expression analysis showed a deregulation of TJ-related pathways in small intestinal mucosa in CD and interestingly, altered expression of several genes including TJP1 (TJ assembly), INADL (apico-basal polarity) and PPP2R3A (negative control of cell growth and division) were not reversed after >2 years on gluten free diet, suggesting genetic involvement. To determine the effect of gliadin and the genetic background on epithelial barrier integrity, we performed functional and expression studies in an intestinal epithelium model, the Caco-2 subclone C2BBe1. Incubation with pepsin-trypsin digested gliadin (PTG) for 4 hours was able to inhibit the formation of cell monolayers but not to disrupt the integrity of those previously formed. When genes crucial for TJ formation were silenced by siRNA, PTG stimulation resulted in the alteration of the TJ pathway and other connected networks, specially Toll-like receptor signaling. Downregulation of central genes in the TJ pathway (TJP1, TJP2 or TJP3) resulted in increased expression of several chemokines. Constitutive altered expression of TJ genes in CD might result in an immature gut barrier that could in turn enhance CD-related gliadin toxicity and result in the activation of proinflammatory cytokine response.

## P07.06

### Ancestry-based stratified analysis of Immunochip data identifies novel associations with celiac disease

*K. Garcia-Etxebarria<sup>1</sup>, A. Jauregi-Miguel<sup>1</sup>, I. Romero-Garmendia<sup>1</sup>, L. Plaza-Izurieta<sup>1</sup>, M. Legarda<sup>2</sup>, I. Iraizorza<sup>2</sup>, J. Bilbao<sup>1</sup>;*

<sup>1</sup>University of Basque Country, Leioa, Spain, <sup>2</sup>Cruces University Hospital, Barakaldo, Spain.

Celiac disease (CD), an autoimmune disorder affecting 1% of the population, is triggered by gluten in genetically susceptible individuals. At least half of the genetic component of CD remains unknown and the functional consequences of associated SNPs are still unclear. To identify candidate genes in CD, we reanalyzed the whole Immunochip CD cohort (139,553 SNPs; 12,041 cases and 12,228 controls) using a new approach that clusters individuals based on immunoancestry prior to disease association analysis, corrected by stratification of those 30 immunogroups. We detected 636 new associated SNPs ( $p < 7.02E-07$ ) located in 5 novel genomic regions. To test whether we could identify putative candidate genes, we performed expression analyses of genes from the top novel region (chr2:134533564-136169524), an extended locus and a gene marked by an isolated SNP, in duodenum biopsies of active and treated CD patients and non-celiac controls. In the novel region, CCNT2 and R3HDM1 were constitutively underexpressed in disease, even after gluten removal. Moreover, several genes within this region were coexpressed in patients, but not in controls. Other novel genes, like KIF21B, REL and SORD showed altered expression in active disease. Apart from the identification of novel CD loci, these results suggest that ancestry-based stratified analysis is a useful approach for association studies in complex diseases.

## P07.08

### Molecular analysis of 29 prevalent translocations and agent of acute leukemia in patients with blood cancer

*F. Ghasemian Sorbeni<sup>1</sup>, E. Sakhinia<sup>2</sup>, A. Esfahani<sup>3</sup>, S. Montazeraheb<sup>4</sup>, N. Karimi ansari<sup>5</sup>, A. Ansarin<sup>5</sup>;*

<sup>1</sup>Department of Genetics Ahar branch, Islamic Azad university, Ahar, Iran, Islamic Republic of, <sup>2</sup>Connective Tissue Disease Research Center, Department of Medical Genetics, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of,

<sup>3</sup>Hematology and Oncology Research Center, Shahid Ghazi Hospital, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of, <sup>4</sup>Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of, <sup>5</sup>Tabriz Genetic Analysis Center (TGAC), Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of.

**Introduction:** Chromosomal translocations are major portion of molecular rearrangement that detected on leukemic subtypes and depending on type of fusion genes, involved in the leukemogenesis. Therefore, accurate screening of this rearrangements is very important in leukemia type identification and appropriate therapeutic approaches.

**Materials and Methods:** To identify chromosomal rearrangements in patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), we used a multiplex RT-PCR protocol in which more than 80 chromosomal breakpoint containing different variants of 29 prevalent translocations were monitored. Of note, a deletion and one duplication, in 8 parallel nested-PCR reactions, in the presence of optimized primers was also assessed. **Results:** In a retrospective analysis on pooled samples from 20 afflicted AML and ALL patients, at least one chromosomal rearrangement was detected by multiplex RT-PCR in 50% of AML and 45% of ALL cases. The efficiency of multiplex RT-PCR method used here was investigated by analysis of available cell lines as positive control for translocations and sequencing of PCR products.

**Conclusions:** Here, we notified that the use of multiplex RT-PCR method could promisingly identify the molecular abnormalities involved during translocation of either AML or ALL patients. Another prominent of this method is that even in low amount of sample can be considered as a potential diagnostic method.

## P07.09

### Identification of congenital blood chimerism in one of the Chinese fraternal twins with mix-filled agglutination in ABO blood grouping

*X. Ye, G. You, J. Wang, Q. Fu;*

*Shanghai Children's Medical Center, Shanghai, China.*

**Introduction:** Chimeras are defined as individuals whose cells are derived from two or more zygotes. Here we report a case of congenital blood chimerism identified by implementing various genotyping technologies in a Chinese patient.

**Materials and Methods:** The patient with ventricular septal defect from Shanghai Children's Medical Center was one of fraternal twins and had no history of transfusion and transplantation. Blood grouping was performed with standard gel centrifugation test cards. ABO genotyping was determined with polymerase chain reaction followed with direct sequencing and clone sequencing. Short tandem repeats analysis was further performed on the blood sample.

**Results:** The patient's red cells were mix-filled agglutination with anti-A antibody, which showed a double populations of O and A red blood cells, whereas his parents were normal type B and type A respectively. Molecular typing showed that normal B101/002 and A102/001 genotypes were identified in his parents respectively, however, the patient exhibited the A102,001,002 three alleles. Moreover, short tandem repeats analysis of the patient revealed three alleles for D5S818,D2S1338,D19S433,vWA,D12S391 and D18S51 loci. We hypothesized that an exchange of blood cells between the fetuses occurred in utero and the additional alleles were contributed by his twin sibling.

**Conclusions:** Using various molecular methods, we identified the blood chimerism in a Chinese patient and provided a basis for further study of this case. Our research also implicated that performing genotyping techniques could reveal the genetic background of blood chimerism and take a deep understanding of the rare case of tetragametic chimerism.

**P07.11****Cytokine Genes Polymorphisms and Susceptibility to Upper Respiratory Tract Infections and Otitis Media in childhood***O. Miljanovic<sup>1</sup>, S. Teofilov<sup>2</sup>, D. Likic<sup>2</sup>, Z. Magic<sup>3</sup>, B. Cikota-Aleksić<sup>4</sup>;**<sup>1</sup>Centre for medical genetic and immunology, Clinical centre of Montenegro, Podgorica, Montenegro, <sup>2</sup>Institute for Public Health of Montenegro, Podgorica, Montenegro,**<sup>3</sup>Institute of Medical Research – Military Medical Academy, Belgrade, Serbia, Belgrade, Serbia, <sup>4</sup>Institute of medical research – Military Medical Academy, Belgrade, Serbia, Beograd, Serbia.*

**Introduction:** Frequent upper respiratory tract infections (URI) and development of susceptibility to URI in childhood, results from complex interactions among host genetic factors, exposure to pathogens, and environmental influences. The goal of this study was to investigate the association of single-nucleotide polymorphisms (SNPs) in cytokine genes and susceptibility to URI in childhood.

**Materials and Methods:** The study enrolled 82 children susceptible to URI and OM and 61 children not susceptible to URI, as a control group, who were exposed to similar environmental/host risk factors (atopic manifestations, day-care attendance, passive smoking, breastfeeding). DNA of all children was studied for specific SNPs in cytokine genes. Genotyping for IL10 1082A→G, IL10 -3575T→A, TNFA -308G→A, IL2 -330T→G and IL6 -597G→A were performed on 7500 Real Time PCR System, using TaqMan pre-designed assays, while genotyping for CD14 -159C→T was performed by using PCR and restriction enzyme digestion with Hae III.

**Results:** The incidence of IL10 -1082 polymorphic genotypes (GA/GG) was significantly higher in children susceptible to URI and OM, than in control group (RR 1.145, 95 % CI 1.011-1.298; p=0.047). Children with high-producing IL10 -1082 (GA/GG) genotypes, exposed to passive smoking, day-care attendance, lack of breastfeeding and with allergic manifestations, were more likely to develop susceptibility to URI and OM.

**Conclusion:** This study pointed out that IL10 -1082 polymorphic genotypes (GA/GG) are associated with increased risk for susceptibility to URI and OM in childhood. Avoidance of environmental risk factors may modify the risk for URI and OM susceptibility children with IL10 -1082 polymorphic genotypes.

**P07.12****Next generation sequencing identifies new candidate genes and variants for idiopathic erythrocytosis and improves diagnostic accuracy***C. Camps<sup>1,2</sup>, N. Petousi<sup>3</sup>, C. Bento<sup>4</sup>, H. Cario<sup>5</sup>, R. R. Copley<sup>1</sup>, M. McMullin<sup>6</sup>, R. van Wijk<sup>7</sup>, WGS500 consortium, P.J. Ratcliffe<sup>3</sup>, P.A. Robbins<sup>8</sup>, J. C. Taylor<sup>1,2</sup>;**<sup>1</sup>Wellcome Trust Centre for Human Genetics, Oxford university, Oxford, United Kingdom,**<sup>2</sup>Comprehensive Biomedical Research Centre, National Institute for Health Research (NIHR), Oxford, United Kingdom, <sup>3</sup>Nuffield Department of Medicine, Oxford university, Oxford, United Kingdom, <sup>4</sup>Hematology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, <sup>5</sup>Department of Pediatrics and Adolescent Medicine, University Medical Center, Ulm, Germany, <sup>6</sup>Centre for Cancer Research and Cell Biology, Queen's University, Belfast, United Kingdom, <sup>7</sup>Department of Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, Netherlands, <sup>8</sup>Department of Physiology, Anatomy and Genetics, Oxford university, Oxford, United Kingdom.*

Erythrocytosis is a rare disorder characterised by increased red cell mass and elevated haemoglobin concentration and haematocrit. Several causative genes have been identified in pathways including oxygen sensing, erythropoiesis and oxygen transport. Despite screening for such genes, in many patients no causative variant is identified. In this study, we combined whole genome sequencing (WGS) and targeted next generation (NGS) sequencing approaches to identify novel genes for idiopathic erythrocytosis.

WGS was performed on samples from 10 idiopathic erythrocytosis patients as part of the WGS500 project resulting in 4 novel candidate genes for erythrocytosis, EPO, GFI1b, KDM6A and BHLHE41 being proposed.

In order to evaluate the likely pathogenicity of these, we sought to replicate these variants by screening a cohort of 125 patients using a custom targeted NGS panel comprising the exonic regions of 21 genes, including the 4 novel genes arising from WGS500, 8 known erythrocytosis genes and 9 others associated with relevant biological pathways.

As a result, 32 exonic rare variants across 36 patients (29%) were detected, of which 10 were known erythrocytosis-causing variants and 22 were novel variants, some with high likelihood of functionality, for which familial segregation, replication and functional studies are underway to further evidence of causality. Of particular interest is the discovery of an additional rare variant in EPO suggesting that this gene should be actively surveyed in idiopathic erythrocytosis patients. Finally we propose that the NGS panel could be used as a clinical tool, to improve accuracy of diagnosis of idiopathic erythrocytosis.

**P07.13****Clinical, genetic and biological characteristics of HbS\_Oman: a severe unrevealed form of sickle cell disease***M. Al Awadi;**Genetics and Developmental Medicine Department, Sultan Qaboos University Hospital, Muscat, Oman.*

**Background:** Hemoglobin SOman results from double mutations in the  $\beta$  globin chain; the classic  $\beta S$  mutation ( $\beta 6$  Glu→Val), and a second mutation in the same chain ( $\beta 121$  Glu→Lys) identical to that of HbOArab. In the literature only 6 carriers have been described. HbS-Oman carriers can have severe clinical presentation matching sickle cell disease.

**Objective:** Study the clinical pheno-genotype, and cell biology of both carriers of HbSOman and compound heterozygotes HbS-SOman.

**Design/Method:** A cross sectional study that includes all identified carriers of HbSOman and compound heterozygotes. Demographic and clinical phenotype data were collected, including family pedigrees that were tracked to the same grandmother indicating a founder effect. Hematological parameters and HPLC were performed.

**Results:** We identified 53 carriers, 27 males and 26 females and 6 HbS-SOman patients, 4 males and 2 females, age range between 2-75 years. Hemoglobin range was 7.5-14.7 g/dl (11.9+1.6), and SOman 9.5-25.3% (15.9+3.7) for carriers, while Hb ranged from 3.2-7.8 g/dl (5.2+2.4) and SOman 6-24.6% (11.6+6.6) for compound heterozygotes. Clinical severity index correlated with the SOman percent in the carriers with a cut-off value of 14% and with decreased production of  $\alpha$ -chains due to deletional or non-deletional mutations; however, there were no such correlations in the compound heterozygotes who presented in early infancy with severe transfusion-dependence. Other genetic modifiers seem to have no effect.

**Conclusion** HbSOman is a severe form of SCD; carriers can be severely symptomatic and compound heterozygotes present as thalassemia major with early transfusion dependence. HSOman cells have increased sickling and hemolytic characteristics.

**P07.14****New Insights into the HLA-G Non-Coding Polymorphisms Affecting Expression Pattern and Clinical Outcome***C. Ribeyre<sup>1</sup>, C. Picard<sup>1,2</sup>, C. René<sup>3</sup>, L. Abi-Rached<sup>4</sup>, P. Gouret<sup>5</sup>, J. Paganini<sup>5</sup>, J. Di Cristofaro<sup>1</sup>;**<sup>1</sup>UMR7268 CNRS-AMU-EFS, Marseille, France, <sup>2</sup>Immuno-genetics laboratory,**<sup>3</sup>Etablissement Français du Sang Alpes Méditerranée, Marsdeille, France, <sup>4</sup>Department of Immunology, CHRU de Montpellier, University Hospital Saint-Eloi, Montpellier, France, <sup>5</sup>Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France, <sup>6</sup>Xegen, Gémenos, France.*

HLA-G is an immunotolerogenic molecule whose expression is modulated in pathological situations (cancers, infectious, inflammatory and autoimmune diseases). Higher HLA-G expression correlates with a reduction in transplant rejection. HLA-G displays a high level of polymorphism in its 5' and 3' untranslated regions, we previously showed that haplotypes formed by these SNPs can predict sHLA-G levels and lung transplantation outcome. Yet, the mechanisms modulating HLA-G expression or clinical outcome are not fully understood. The aim of this study is to predict transcription factor binding sites each haplotype. To fully characterize HLA-G variation in coding and untranslated regions, we typed 500 healthy individuals by NGS. Data were analyzed by the Xegen Company. Using all SNPs, including 19 SNPs in transcription factor-binding sites, we could define 35 haplotypes, 24 with estimated frequencies below 0.3%, and 11 with estimated frequencies above 1%, the latter representing 96% of the 1,000 haplotypes investigated and their structure was similar to previously observed. Analysis of transcription factor-binding site content revealed that all haplotypes carry multiple sites for SP1. Similarly, while all haplotypes display a progesterone receptor-binding site, a mutation in this region characterizes the haplotype associated with poor lung transplantation outcome. Other haplotypes displayed specific binding sites for Oct-1, C/EBP $\alpha$ , TEF-1, Elk or STAT. These results support that NGS technology offers a pertinent solution for complete HLA analysis in clinical context and that nucleotide variations in transcription factor-binding sites can account for the modulation of HLA-G expression and clinical outcomes.



**P07.15**

### Whole Exome Sequencing in a large cohort of patients affected with Inherited Thrombocytopenia

*C. Marconi<sup>1</sup>, R. Bottega<sup>2</sup>, B. Serena<sup>3</sup>, F. Palombo<sup>1</sup>, V. Bozzi<sup>2</sup>, M. Faleschini<sup>4</sup>, D. DeRocco<sup>2</sup>, C. Cagioni<sup>3</sup>, C. Gnan<sup>2</sup>, P. Noris<sup>5</sup>, A. Savoia<sup>4,2</sup>, C. L. Balduini<sup>3</sup>, A. Pecci<sup>3</sup>, T. Pippucci<sup>1</sup>, M. Seri<sup>1</sup>;*

<sup>1</sup>*University of Bologna -Department of Medical and Surgical Sciences, Bologna, Italy;* <sup>2</sup>*Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Trieste, Italy;*

<sup>3</sup>*Department of Internal Medicine, University of Pavia-IRCCS Policlinico San Matteo Foundation, Pavia, Italy;* <sup>4</sup>*Department of Medical Sciences, University of Trieste, Trieste, Italy;* <sup>5</sup>*Department of Internal Medicine, University of Pavia-IRCCS Policlinico San Matteo Foundation, P, Italy.*

Inherited Thrombocytopenias (IT) are a group of heterogeneous disorders caused by almost 30 genes. Although many genes have been identified since the introduction of Whole Exome Sequencing (WES), almost half of IT patients still remains without a molecular diagnosis. As IT are characterized by a wide genetic heterogeneity, identification of novel disease-genes is still challenging. In our cohort of 274 affected families characterized at the molecular level, 5 genes are responsible for about 50% of cases, while the remaining genes are responsible each for less than 2% of cases. To overcome this difficulty and describe novel IT genes, we performed WES in 107 patients (86 families) affected with autosomal dominant IT of unknown molecular origin. We compared the cumulative variant allele frequencies (sum of the allele frequencies of each variant) of each gene in our IT cases to that of a control cohort, consisting of the Exome Aggregation Consortium non-Finnish European samples (33.370 individuals) and we found 7 genes, affecting 22 families, significantly enriched in rare variants ( $f < 1 \times 10^{-5}$ ) in IT cases. Two genes (ACTN1, ETV6) are known to be causative for IT. The identified variants was confirmed to be pathogenic through segregation analysis and functional studies. For the other 5 genes, which are novel candidate genes, segregation analysis and screening of further IT patients are ongoing. Conclusions: the availability of a large patients cohort can overcome the problems due to genetic heterogeneity and led to the identification of 5 strong candidate IT genes in our cohort.

**P07.16**

### Evaluation of Micro particle Production by JAK2V617F positive the Endothelial Cells

*H. Hekimoglu, I. Uslu, S. Sözer Tokdemir;*

*Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey.*

Introduction: A cytoplasmic tyrosine kinase (JAK), is encoded by Janus Kinase 2 gene (JAK2), plays an important role in growth and differentiation of hematopoietic cells. A point mutation, characterized as V617F, is found mostly in Philedelphia-chromosome negative myeloproliferative neoplasms (MPN). Recently, micro particles (MP) were related with the thromboembolic complications. This study focuses on the role of JAK2<sup>V617F</sup> in MP production from endothelial cells (EC).

Materials and Methods: Lentiviral vectors, carrying JAK wild type or mutant in addition to green fluorescent protein (GFP), were transfected to Human Embryonic Kidney cells by Lipofectamine. The viral particles were collected by ultracentrifugation and infected into Human Umbilical Vein EC. In order to isolate MPs, the infected EC culture supernatants were centrifuged and concentrated. The spectrophotometric evaluation of the MP was performed. After labeling of MPs with CD31, the flow-cytometry analysis were performed with applying 0.5-0.9  $\mu$ m microbeads to set up MP gates.

Results: In this study, we have successfully infected the lentiviral vectors into EC. The analysis of the infected EC supernatants was demonstrated that the MPs were composed of RNAs. Labeling the MPs with EC marker CD31 revealed the origin MPs to be EC and both JAK2 and JAK2<sup>V617F</sup> increased the MP production 2 fold compared to GFP control.

Conclusion: This study provides insight to understand the possible effects of JAK2<sup>V617F</sup> on EC regarding to the MP production. Further studies might be helpful to understand the role of JAK2<sup>V617F</sup> on EC and as well as the role of MPs for thrombus formation.

**P07.17**

### Resolving KIR genotypes and haplotypes simultaneously using Single-Molecule, Real-Time Sequencing

*J. M. Ekholm<sup>1</sup>, C. W. Pyo<sup>2</sup>, K. Eng<sup>1</sup>, R. Hall<sup>1</sup>, C. Vierra-Green<sup>3</sup>, S. Spellman<sup>3</sup>, D. Roe<sup>4</sup>, D. E. Geraghty<sup>4</sup>, M. Maier<sup>4</sup>, S. Ranade<sup>1</sup>;*

<sup>1</sup>*PacBio, Menlo Park, CA, United States;* <sup>2</sup>*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States;* <sup>3</sup>*Center for International Blood and Marrow Transplant Research, Minneapolis, MN, United States;* <sup>4</sup>*National Marrow Donor Program, Minneapolis, MN, United States.*

The killer immunoglobulin-like receptors (KIR) genes belong to the immunoglobulin superfamily and are widely studied because of the critical role

they play in coordinating the innate immune response to infection and disease. Resolution of the complete KIR repertoire remains challenging as the region is repetitive and extremely polymorphic, both on the population and sequence level. All together, the KIR genes cluster spans  $\sim 150$  kb of chromosome 19q13.4.

Highly accurate, contiguous, long reads, like those generated by SMRT Sequencing, when combined with target-enrichment protocols, provide a straightforward strategy for generating complete de novo assembled KIR haplotypes. Here we generated complete diploid haplotypes of the KIR region for 8 individuals on the PacBio RS II platform. The haplotypes ranged in size from 75 kb to 250 kb and captured complete, phased sequence information, with one exception in which a gap of 1 kb was found. This particular example was later determined to be the largest and most complex haplotype in the cohort. Large structural variations within the region were also observed, with two haplotypes showing deletions spanning both the centromeric and telomeric regions and one other haplotype containing a large tB01 insertion.

These data represent the first demonstration of de novo assembled KIR haplotypes derived from diploid human genomes using single-molecule sequencing. The resulting sequences demonstrate the extensive diversity and heterogeneity of the KIR region, which makes investigation into this locus uniquely suited for long-read single-molecule sequencing.

**P07.18**

### The characterization of KMT2A (MLL) chromosomal breakpoints

*M. Macejova<sup>1</sup>, L. Zejskova<sup>1</sup>, R. Plachy<sup>1</sup>, T. Jancuskova<sup>1</sup>, D. W. Hardekopf<sup>2</sup>, J. Stika<sup>1</sup>, M. Ondrackova<sup>1</sup>, N. Kosyakova<sup>2</sup>, T. Liehr<sup>2</sup>, A. Zavrelova<sup>3</sup>, P. Zak<sup>3</sup>, V. Petecukova<sup>4</sup>, J. Novak<sup>4</sup>, T. Kozak<sup>4</sup>, S. Pekova<sup>1</sup>, S. Pekova<sup>1</sup>, M. Karas<sup>5</sup>;*

<sup>1</sup>*synlab genetics s.r.o, Prague, Czech Republic;* <sup>2</sup>*Institute of Human Genetics, Jena University Hospital, Jena, Germany;* <sup>3</sup>*4th Department of Internal Medicine – Hematology, Charles University Hospital and Faculty of Medicine, Hradec Kralove, Czech Republic;*

<sup>4</sup>*Internal Clinic of Hematology University Hospital Kralovske Vinohrady, Prague, Czech Republic;* <sup>5</sup>*Department of Hemato-oncology, University Hospital Plzen, Plzen, Czech Republic.*

Introduction: Chromosomal rearrangements involving the human KMT2A gene (MLL, mixed lineage leukemia) are associated with development of acute leukemia. The presence of certain MLL rearrangements is an independent prognostic factor and patients are usually treated according to high-risk protocols. Minimal residual disease (MRD) detection provides an objective assessment of treatment response. However, MRD assays using MLL fusion transcripts as molecular markers usually do not provide sufficiently sensitive detection of residual leukemic cells.

Methods: For the identification of MLL breakpoint sequences we used two different approaches. First approach was long-range PCR followed by sequencing of the PCR products. Second approach included combination of conventional chromosome microdissection, next-generation sequencing and sequencing of the final PCR products. Leukemia/patient-specific sequences of the chromosomal breakpoint were used to develop MRD assays and enabled us to perform sensitive monitoring of MRD using qPCR in 9 acute leukemia patients.

Results: We identified unique breakpoint sequences in 9 patients with acute leukemia. Using first approach we detected MLL/AF6, MLL/AF9 (2 patients), MLL/AF10, MLL/ENL (2 patients) and MLL/ELL. Second approach was used for identification of MLL/AF10 and MLL/AF4 breakpoints. Identification of unique breakpoint sequences was followed by the design of sufficiently sensitive MRD assays. The MRD levels of residual leukemic cells correlated with clinical outcome.

Conclusion: MLL breakpoints could be identified by various methods (inverse PCR, panhandle PCR). Our results show other approaches for identification of unique MLL breakpoint sequences, which can be utilized for the design of the leukemia-specific assay for MRD monitoring in patients with acute leukemia.

**P07.19**

### The role of partially methylated domains in normal and neoplastic hematopoietic cells

*A. Merkel<sup>1</sup>, M. Duran Ferrer<sup>2</sup>, J. I. Martin-Subero<sup>2</sup>, S. C. Heath<sup>1</sup>, the BLUEPRINT consortium;*

<sup>1</sup>*Centro Nacional d'Analisi Genomica (CNAG-CRG), Barcelona, Spain;* <sup>2</sup>*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.*

Leukemia is the 12th most common cancer in Europe, affecting approximately 13 in 100000 people. As part of the European epigenome project BLUEPRINT, we have recently conducted a survey of DNA methylation in over 170 samples from diverse hematopoietic cell types including various leukemia subtypes.

Globally, we find that cells of the lymphoid lineage modulate their methylation more than those of the myeloid lineage. Although many sites of dynamic DNA methylation coincide with tissue specific regulatory regions, a significant proportion (particularly in terminally differentiated lymphoid cells) is associated with large partially methylated domains (PMDs) - a tendency that is amplified in the related malignancies.

They appear compact heterochromatin, associated with repressive histone marks, low transcriptional activity and late replication timing. At the same time, these regions coincide with active regions of open chromatin in embryonic stem cells, indicating that they might have originated from areas under 'lockdown' from the pluripotent state to ensure cell fate.

We propose that PMDs represent higher order chromatin structures that are established early during lymphoid differentiation as part of lineage commitment. Over time and with increasing number of cell replications these regions experience passive loss of methylation ultimately forming large domains of low and intermediate methylation. We find that in lymphoid leukemias these regions are further enlarged and contribute to low global methylation, presumably caused by the high proliferation rate that is a hallmark of most cancers. As such, we believe the chromatin structures underlying PMDs are of significant importance for cell identity and integrity.

#### P07.20

##### **MonoMac syndrome, a rare cause of neutropenia**

**M. T. Bataneant, Maria Puiu, Patricia Urtila, Mihaela Baica, Mihaela Lelik, Maria Pop, Margit Serban, M. Puiu, P. Urtila, M. Baica, M. Lelik, M. Pop, M. Serban;**  
University of Medicine and Pharmacy, Timisoara, Romania.

**Introduction.** Congenital neutropenias usually are characterized by bacterial recurrent severe infections but chronic viral infection is not a part of the clinical picture of this group of rare diseases.

**Case presentation.** A male without significant family history of chronic infections or malignancy, who had at 4 years of age generalized warts with an evolution of 4 years was diagnosed accidentally at 11 years of age with mild neutropenia(700/mmc) and mild decreased platelet count(130.000/mmc). Bone marrow aspiration showed normal cellularity without any dysplastic feature. He was treated with Prednison and G-CSF without response. Secondary causes of neutropenias were excluded by absence of drug administration history, negative serology for HBV, HCV, HIV, CMV, EBV and negative ANA, dsDNA, ANCA antibodies. Immunologic tests showed IgA=0,46g/l(0,7-4,32), IgG=6 g/l(6,44-11,96), IgM=2,12 g/l(0,52-3,57), BLf=270(300-500), TLF=2548(1400-2000), CD4+=1093(700-1100), CD8+=1381(600-900), NK=79(200-300). WHIM syndrome was suspected but no mutation in CXCR4 gene was founded. Vinh described in 2010 a new disease-MonoMac syndrome, characterized by monocytopenia with mycobacterial and viral infection caused by mutation in GATA2 gene. Reanalyzing the hemoleogram we founded persistent decreased count of monocyte cells < 100/mmc. The genetic test identified mutation c.1192 C>T R398W in GATA2 gene.

**Conclusion:** Even if neutropenia is not a part of the clinical definition of the MonoMac syndrome, we must think to it when neutropenia is associated with chronic viral infection and low count of monocyte cell. 50% of these patients will develop myelodysplastic syndrome and myeloblastic leukemia, so bone marrow transplantation is indicated before developing of these complications.

#### P07.21

##### **Skin lesions and arthralgias in a large pedigree with a partially anakinra-responsive and possibly IL-18 driven NLRC4-related autoinflammatory disease**

**N. M. L. Volker-Touw<sup>1</sup>, H. D. de Koning<sup>2</sup>, T. van Kempen<sup>3</sup>, K. Oberndorff<sup>4</sup>, J. Giltay<sup>1</sup>, C. G. F. de Kovel<sup>1</sup>, M. Boes<sup>5</sup>, M. A. M. van Steensel<sup>6</sup>, G. T. J. van Well<sup>7</sup>, A. Simon<sup>8</sup>, J. Frenkel<sup>9</sup>, M. E. van Gijn<sup>1</sup>;**

<sup>1</sup>Department of Genetics, University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>Dept. of Dermatology, Radboud University Medical Center, Nijmegen, Netherlands, <sup>3</sup>Dept. of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, Netherlands, <sup>4</sup>Dept. of Pediatrics, Atrium-Orbis Medical Centre, Sittard, Netherlands, <sup>5</sup>Dept. of Pediatric Immunology and Laboratory of Translational Immunology (LTI), University Medical Center Utrecht, Utrecht, Netherlands, <sup>6</sup>Dept. of Dermatology, Maastricht University Medical Center, Maastricht, Netherlands, <sup>7</sup>Dept. of Pediatrics, Maastricht University Medical Center, Maastricht, Netherlands, <sup>8</sup>Dept. of General Internal Medicine, Radboud University Medical Center, Nijmegen, Netherlands, <sup>9</sup>Dept. of Pediatrics, University Medical Center Utrecht, Utrecht, Netherlands.

Autoinflammatory disorders (AID) are characterized by chronic or recurrent systemic sterile inflammation. It is a genetically heterogeneous group of diseases. Recently, gain of function mutations in *NLRC4* have been described to be associated with AID.

We performed whole exome sequencing in the members of a large 6 generation pedigree with a hitherto unknown AID. Medical data were collected retrospectively. IL-1 $\beta$  and IL-18 concentrations were analyzed in plasma. Skin biopsies were obtained of lesional and uninvolved skin from three patients for routine histology and immunohistochemical staining of IL-1 $\beta$  and IL-17. Exome sequencing revealed a novel heterozygous c.1333T>C p.(Ser445Pro) variant in *NLRC4*. The variant segregated with the 13 affected family members (LOD-score 3.58). Prediction software programs (Sift, Polyphen) indicated pathogenic properties. Moreover the variant is located next to the recently described p.(His443Pro) pathogenic mutation. In affected family members, the clinical phenotype was influenced by weather conditions, stress, and infection. Severity of the clinical phenotype varied considerably. In a subset of the patients, the clinical symptoms resolved promptly after anakinra treatment, while other patients responded only partially. Biopsies of lesional skin showed a unique lymphocytic-histiocytic infiltrate in the dermis, unlike the histology of previously described autoinflammatory skin disease. Plasma concentrations of IL-18 were extremely elevated in all patients, even without an inflammatory episode (median 4,324 pg/ml; ref. 0-34 pg/ml).

In conclusion, in this study we describe a novel variant in *NLRC4* in a large pedigree with a partially anakinra-responsive AID, and expanded the clinical phenotype associated with *NLRC4*-inflammasome mediated AID.

#### P07.22

##### **Physiological repression of PARP1 transcription during pro-inflammatory macrophages development results from differentiation-associated cell cycle arrest in G0/G1 phase**

**A. Robaszkiewicz, E. Wisnik;**

*University of Lodz, Institute of Biophysics, Department of Environmental Pollution Biophysics, Lodz, Poland.*

ADP-ribosylation - post-transcriptional modification of proteins carried out by PARP1 is capable of shifting gene expression up and down. In human pro-inflammatory macrophages (M1) PARP1 acts as a repressor of genes encoding cytokines and as indicated in our study the differentiation process of CD34+ hematopoietic stem cells and premonocytic cell line THP1 into macrophages is associated with the loss of PARP1. Therefore, we searched for the mechanism responsible for PARP1 repression during pro-inflammatory macrophage development. The differentiation of HSC and THP1 was induced with GM-CSF and PMA, respectively. The expression of PARP1 was determined with real-time PCR and western blot. The contribution of miRNA was verified using Ago2 inhibitor - aurintricarboxylic acid, RNA synthesis was monitored with nascent RNA capturing, cell cycle was analyzed with flow cytometry. Chromatin association with transcription factors was monitored with ChIP.

The reduction of PARP1 protein in macrophages results from transcriptional repression of PARP1 gene as these cells demonstrate lower Pol2 occupancy around TSS and de novo synthesis of PARP1 mRNA. Moreover, Ago2 inhibitor failed to rescue PARP1 decrease indicating that miRNA does not contribute to mRNA degradation. The treatment of proliferating macrophage precursors with mimosine, which mimicks differentiation-induced cell cycle arrest in G0/G1 phase, triggered comparable decrease in PARP1 expression. Further analysis revealed the cell cycle-dependent modulation of Sp1 interaction with PARP1 promoter as a mechanism responsible for the differentiation-induced PARP1 transcriptional repression.

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#### P07.23

##### **A Phelan-McDermid syndrome case with immunological findings**

**A. G. Zaman<sup>1</sup>, E. H. Sayar<sup>2</sup>, S. G. Güner<sup>2</sup>, M. S. Yıldırım<sup>1</sup>, & Reisli<sup>2</sup>;**

<sup>1</sup>Necmettin Erbakan University, Meram Medical Faculty, Department of Medical Genetics, Konya, Turkey, <sup>2</sup>Necmettin Erbakan University, Meram Medical Faculty, Department of Pediatric Immunology, Konya, Turkey.

Phelan-McDermid syndrome (PHMDS) can be caused by a heterozygous contiguous gene

deletion at chromosome 22q13. At least 500 cases of 22q13.3 deletion syndrome are known. Characteristic signs and symptoms of the syndrome include normal to accelerated growth, moderate to profound intellectual disability, decreased muscle tone (hypotonia), and absent to severely delayed speech.

A 15-year-old speechless, mentally deficient, unable to walk male underwent genetic evaluation. He had no eye contact. Clinical examination showed a small forehead, long eyelashes, epicanthal folds and lowset ears, large and broad hands and feet with short terminal phalanges. The brain

MRI indicated thinning of the corpus callosum (between the body and the splenium), widespread cortical atrophy and dilation of the lateral ventricles, mild widespread reduction of the white matter. We can not get metaphase peripheral blood culture. Then cytogenetic analysis were performed from fibroblast cultures. FISH was performed with DiGeorge/VCFS Probe TUPLE1(22q11.2)/ARSA control probe (22q13.3) (Cytocell,UK). The ARSA (control) probe (22q13.3) did not hybridize and the diagnosis of the 22q13 deletion syndrome was confirmed. Although there is no history of frequent infections, all of immunoglobulin levels, except IgE, revealed to be markedly decreased. Besides, lack of memory B cells, total T and helper T lymphocyte ratio were also found lower than normal from immunological analysis of peripheral lymphocyte by flow cytometry.

In this report, the remarkable clinical and immunologic findings were presented and discussed in detail.

## P07.24

### Exome sequencing of 160 patients with primary immunodeficiencies: translational genomics with direct clinical implications

P. Arts<sup>1</sup>, A. Simons<sup>1</sup>, E. Yilmaz<sup>2</sup>, A. R. Mensenkamp<sup>1</sup>, W. A. van Zelst-Stams<sup>1</sup>, M. van Deuren<sup>3</sup>, M. van der Flier<sup>4</sup>, A. Simon<sup>3</sup>, J. A. Veltman<sup>1</sup>, F. L. van de Veerdonk<sup>2</sup>, A. Hoischen<sup>1</sup>, M. G. Netea<sup>3</sup>;

<sup>1</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>2</sup>Department of Medical Biology, Faculty of Medicine, Akdeniz University, Antalya, Turkey, <sup>3</sup>Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, Netherlands, <sup>4</sup>Department of Pediatric Infectious diseases, Department of Pediatrics, Radboud University Medical Center, Nijmegen, Netherlands.

**Introduction:** Primary immunodeficiencies (PIDs) are severe medical conditions caused by non-redundant defects of innate or adaptive immune responses, leading to recurrent, severe infections. Severely affected PID patients with an unknown genetic defect were selected for diagnostic exome-sequencing.

**Methods:** 160 PID cases were included in this study. First, variation in 294 genes known to cause PIDs are studied using an *in silico* filter during exome data-analysis. Next, 102 patients without a genetic diagnosis after step 1 gave permission for exome-wide analysis of the data. In selected cases *in vitro* stimulations of identified cytokine production capacity defects and provided additional functional evidence for the diagnosis.

**Results:** Diagnostic exome analysis revealed (likely) pathogenic mutations in known PID genes in 32 cases (20%). Exome-wide analysis led to identification of additional causative mutations in 10 cases, for which underlying genes have a known role in specific immunological pathways. One example with direct clinical implication of this approach was the characterization of the genetic, and functional defects in 3 patients with autoimmune lymphoproliferative syndrome type V and (*de novo*) *CTLA4* mutations. Several studies suggest abatacept as treatment for patients with *CTLA4* deficiency. **Conclusion:** Exome sequencing can provide a genetic diagnosis in a significant number of patients with PIDs. It is important to perform exome-wide analyses after an analysis of the known PID genes because many new PID genes are only just being discovered. The molecular diagnosis provided novel insight in therapeutic options for a subset of our patients.

## P07.25

### Enrichment of rare disease causing variants in population isolates: the Finnish Disease Heritage example

L. Trott<sup>1</sup>, T. Hautala<sup>2</sup>, S. Hämäläinen<sup>3</sup>, J. Syrjänen<sup>4</sup>, H. Viskari<sup>4,5</sup>, M. Lepistö<sup>1</sup>, K. Heiskanen<sup>6</sup>, M. Taskinen<sup>7</sup>, K. Porkka<sup>8</sup>, A. Palotie<sup>1,9</sup>, M. Seppänen<sup>10,11</sup>, J. Saarela<sup>1</sup>;

<sup>1</sup>Institute for Molecular Medicine Finland (FIMM), Helsinki University, Helsinki, Finland,

<sup>2</sup>Department of Internal Medicine, Oulu University Hospital, Oulu, Finland, <sup>3</sup>Department of Internal Medicine, Tampere University Hospital, Tampere, Finland, <sup>4</sup>Department of Virology, University of Tampere, School of Medicine, Tampere, Finland, <sup>5</sup>Children's Hospital, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland,

<sup>6</sup>Helsinki University Hospital, Helsinki, Finland, <sup>7</sup>Hematology Research Unit, Biomedicum Helsinki and Hematology Division, Comprehensive Cancer Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>8</sup>Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>9</sup>Rare Disease Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>10</sup>Adult Immunodeficiency Unit, Inflammation Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland.

The correlation between the genetic structure of the population and the distribution of genetic disorders supports a population perspective in medical genetics, especially in the field of rare monogenic disorders. The benefits are even higher if applied to population isolates.

Finland presents a characteristic population history with a restricted number of founders, isolation, several population bottlenecks, and recent expansion of the population. This has led to the enrichment of some deleterious variants and loss of others, creating a phenomenon called the Finnish Disease Heritage (FDH).

By definition, FDH disorders are more frequent in Finland than elsewhere. Within each disorder the majority (if not all) of the Finnish patients share the same founder *Fin<sub>major</sub>* mutation, again over-represented in Finland. Nonetheless, FDH disorders are rare (1:10,000-1:100,000). Today, FDH comprises 36 disorders (32 autosomal recessive, two autosomal dominant, and two X-chromosomal) with a disease spectrum spreading to all branches of medicine, but more consistently in pediatrics. Some FDH disorders can be effectively treated, assuming the correct diagnosis has been performed.

Investigating by whole-exome sequencing the molecular defects behind rare immune disorders in Finnish patients, we identified rare homozygous FDH *Fin<sub>major</sub>* variants in the *CUBN* (megaloblastic anemia) and *RECQL4* genes (Rapadilino syndrome).

In addition, we found a previously known disease-associated *AICDA* variant causing primary antibody deficiency (HIGM2) as part of a founder haplotype. The variant is strongly enriched in Finland (~38.5 fold) compared to other populations with European ancestries, suggesting it as a potential *Fin<sub>major</sub>* mutation and HIGM2 as a newly found FDH disorder.

## P07.26

### Functional study of Peptidylarginine deiminase type 4 as a genetic risk factor for Rheumatoid arthritis

A. Suzuki<sup>1</sup>, Y. Kochi<sup>1</sup>, H. Matsumura<sup>1</sup>, E. Kanno<sup>1</sup>, K. Yamamoto<sup>1,2</sup>;

<sup>1</sup>RIKEN, Yokohama, Japan, <sup>2</sup>Univ. of Tokyo, Tokyo, Japan.

Rheumatoid arthritis (RA) patients frequently have autoantibodies to citrullinated peptides, suggesting the involvement of the citrullinating enzymes, peptidylarginine deiminases (PADIs), in RA. Previous linkage studies showed that a RA-susceptibility locus included four PADI genes, however it was not clear which PADI gene confers RA susceptibility. Peptidylarginine deiminase type 4 (PADI4) was identified as a susceptibility gene for RA in a trans-ethnic meta genome-wide association study (GWAS). PADI4 is one of the PADI gene family and converts arginine residue to citrulline residue. PADI4 is highly expressed in immune tissues and cells, especially bone marrow, macrophages, neutrophils and monocytes. Peptidylcitrulline is an important molecule in RA, because it is an antigen of ACPA and only PADI enzymes (translated protein from PADI genes) can provide peptidylcitrullines by modification of protein substrates. To understand the importance of PADI4 gene in the progression of RA, we used Padi4<sup>-/-</sup> mice and showed that PADI4 is significantly affected to progress of collagen induced arthritis (CIA) RA model mice. In Padi4<sup>-/-</sup> CIA mice sera, the concentrations of serum anti-CII IgM, IgG, and levels of inflammatory cytokines decreased significantly rather than in WT CIA mice. We demonstrated that Padi4 expression was induced by CII immunization. In Padi4<sup>-/-</sup> mice, inflammatory cytokine levels were significantly decreased compared with those in wild-type mice. Interestingly, Padi2 expression was induced in immune cells of Padi4<sup>-/-</sup> mice in compensation for the defect in Padi4. As the results, we suggested that Padi4 enhanced collagen-initiated inflammatory responses.

## P07.27

### Newborn screening for severe combined immunodeficiency (SCID) using T-cell receptor circles (TRECs) and high-throughput sequencing (HTS)

J. M. Strand<sup>1</sup>, K. Gul<sup>2</sup>, T. G. Abrahamsen<sup>2,3</sup>, E. Lundman<sup>1</sup>, H. Gaup<sup>1</sup>, H. Erichsen<sup>2</sup>, T. Øverland<sup>2</sup>, A. Rønnestad<sup>2</sup>, T. E. Hartmann Bjørndalen<sup>4</sup>, H. S. Sorte<sup>5</sup>, T. Tangeraas<sup>1,2</sup>, J. Jørgensen<sup>1</sup>, C. L. Salvador<sup>1,6</sup>, A. D. Rowe<sup>1,6</sup>, T. Rootwelt<sup>1,2</sup>, R. D. Pettersen<sup>1</sup>, A. Stray-Pedersen<sup>1</sup>;

<sup>1</sup>Norwegian National Unit for Newborn Screening, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Department of Paediatrics, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway, <sup>3</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway, <sup>4</sup>Department of Obstetrics and Gynecology, Women's Division, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway, <sup>5</sup>Department of Medical Genetics, Oslo University Hospital, Oslo, Norway, <sup>6</sup>Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway.

**Introduction:** Children with SCID are apparently healthy at birth, but may acquire life-threatening infections during first months of life. Newborn screening (NBS) for SCID was started as a prospective research project based on informed, written consent in 2015. We are also conducting a retrospective study using samples from patients with known immunodeficiencies to test cut-off values and 2nd tier HTS sequencing.

**Materials and Methods:** TRECs are analyzed by RT-PCR in DNA extracted from the same dry blood spot card as the ordinary NBS program. Cut-off was set to 40 TREC/μl, and only samples under cut-off and with normal β-actin

levels are positive on the 1st tier. 2nd tier testing of known immunodeficiency genes using the IonAmpliSeq PID panel on Ion Torrent PGM is performed to increase positive predictive value.

Results: Of the 720 children tested so far, the average level is 250 TREC/µl. 11 samples, all from NICU patients, have fallen below cutoff, without suspicion of a SCID diagnosis. In the retrospective project, screening samples from 7 patients with known immunodeficiency due to defects in IL2RG, JAK3, IL7R, PGM3, LIG4, RECQL4 and ADA had TRECs of 0, 0, 0, 11, 0, 60, and 0/µl, respectively. 2nd tier HTS was able to confirm the molecular alterations in 5/5 of the 7. The PID gene panel lacked coverage of the remaining mutations, and needs refinement before implementation.

Conclusion: TRECs as 1st tier, and HTS SCID gene panel as 2nd tier, promise rapid detection of newborns with SCID and other severe T-cell deficiencies.

#### P07.28

#### High-throughput multiplexed digital droplet PCR for detection of severe combined immunodeficiency in newborn screening

*N. Vidal-Folch, D. Milosevic, R. Majumdar, D. K. Gavrilov, D. Matern, K. Raymond, P. Rinaldo, S. Tortorelli, R. S. Abraham, D. Oglesbee;*  
Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN, United States.

Introduction: Measurement of T-cell receptor excision circles (TREC) is widely used for detection of Severe Combined Immunodeficiency (SCID) in newborn screening programs (NBS). Currently, qPCR is predominantly used to quantify TREC in dried blood spots (DBS). qPCR requires normalization to controls and is susceptible to variations in amplification efficiency. We evaluated digital droplet PCR (ddPCR) as a novel method for SCID detection.

Materials and Methods: DNA was extracted from one 3mm DBS punch using wash steps and denaturation at 99°C. Simultaneous quantification of TREC and RNaseP (internal control) was performed using AutoDG and QX200 ddPCR Bio-Rad system. A total of 10 diagnosed patient DBS (1 X-linked SCID, 1 RAG1 Omenn syndrome, 2 idiopathic T-cell lymphopenia (iTCL), 1 chr22q11.2 deletion negative TCL, 2 DiGeorge syndrome, 1 CHARGE syndrome, 1 ataxia telangiectasia and 1 cartilage hair hypoplasia) and 80 normal DBS (age≤7days, gestational age≥36weeks) were screened for TREC levels. Results are expressed in TREC/µl blood.

Results: All previously diagnosed patient DBS were confirmed to contain ≤15 TRECs. Normal DBS ranged 46-276, average of 132 TRECs. Dilution experiments showed LLOQ at 14 TRECs. LOD was 11 TRECs. Precision experiments showed <20 %CV for intra-assay (at 54 TRECs) and inter-assay (at 60 TRECs) reproducibility. A reference range study is currently ongoing.

Conclusions: We have developed a highly sensitive and accurate multiplexed ddPCR method for absolute quantification of TREC in DBS without the need for standard curve. The method is cost-effective, high-throughput and suitable for NBS testing.

#### P07.29

#### Altered expression of DNA repair enzymes involved in molecular signaling pathway dependent on p53 in systemic lupus erythematosus

*M. D. Olivares<sup>1,2</sup>, J. Perez-Hernandez<sup>1,2</sup>, V. Lendinez-Tortajada<sup>1</sup>, M. J. Forner<sup>2</sup>, F.J. Chaves<sup>1,3</sup>, J. Redon<sup>2,4</sup>, R. Cortes<sup>1,2</sup>;*  
<sup>1</sup>Genotyping and Genetic Diagnosis Unit, INCLIVA Biomedical Research Institute, Valencia, Spain, <sup>2</sup>Cardiometabolic and Renal Unit, INCLIVA Biomedical Research Institute, Valencia, Spain, <sup>3</sup>CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Institute of Health Carlos III, Barcelona, Spain, <sup>4</sup>CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Madrid, Spain.

Introduction: In systemic lupus erythematosus (SLE) apoptosis is thought to be defective, producing increased extracellular DNA and dsDNA antibodies. The p53 pathway is crucial in apoptosis regulation. We investigated if expression of enzymes (OGG1 and SIRT1) and transcription factor (FOXO1) involved in p53 pathway were altered in SLE, and associated with immune activation.

Material and Methods: We studied 32 SLE patients (10 with active lupus nephritis (LN), 12 with inactive LN, and 10 SLE in absence of LN); and 20 healthy controls. The mRNA levels for p53, SIRT1, OGG1 and FOXO1 were quantified by RT-qPCR in the urinary cellular pellet.

Results: Urinary mRNA levels of p53, OGG1 and FOXO1 were significantly diminished in active LN ( $p<0.01$ ,  $p<0.01$  and  $p<0.05$ , respectively), whereas in the other patient groups were similar to control group. However, the expression of SIRT1 in the urinary sediment had a significant increase ( $p<0.01$ ) in active group compared to controls. Furthermore, the three patient groups showed an inverse correlation between FOXO1 and OGG1 mRNA levels with antibodies anti-dsDNA ( $r=-0.47$  and  $r=-0.55$ ,  $p<0.01$ ), and Systemic Lupus Activity Index (SLEDAI) ( $r=-0.22$  and  $r=-0.41$ ,  $p<0.05$ ). Whereas urinary expression of SIRT1 had a direct correlation with anti-dsDNA ( $r=0.87$ ,

$p<0.001$ ) in active LN.

Conclusions: These data show a transcriptional dysregulation in DNA repair enzymes SIRT1 and OGG1, and transcription factor FOXO1 involved in the p53 molecular pathway in SLE patients. In addition, mRNA quantification in the urinary sediment could be a non-invasive method to establish its association with the renal impairment in SLE.

#### P07.30

#### Screening of KLF1 gene variants in cases with increased levels of fetal hemoglobin

*A. Karamanli<sup>1</sup>, C. Chassanidis<sup>2</sup>, E. Boutou<sup>2</sup>, E. Voskaridou<sup>3</sup>, A. Balassopoulou<sup>2</sup>;*  
<sup>1</sup>School of Biology, National and Kapodistrian University of Athens, Athens, Greece,  
<sup>2</sup>Molecular Genetics Laboratory, Thalassaemia and Hemoglobinopathies Center, Laiko General Hospital, Athens, Greece, <sup>3</sup>Thalassaemia and Hemoglobinopathies Center, Laiko General Hospital, Athens, Greece.

Introduction: KLF1 is a transcription factor, promoting shift in expression from  $\gamma$  to  $\beta$ -globin. This process is carried out either directly, by activating transcription of  $\beta$ -gene, or indirectly by activating transcription repressors for silencing  $\gamma$ -genes. Variations of KLF1 lead to increased expression of fetal hemoglobin (Hb F), showing clinical benefits for thalassaemia patients, making it an ideal target for gene intervention to increase HbF levels and thus ameliorating the patient's phenotype.

Material and Methods: This study aimed to identify possible variations of KLF1 gene, in  $\beta$ -thalassaemia heterozygotes and normal controls presenting high levels of HbF>4%. DNA from 47 blood samples was obtained (32 heterozygotes/ 15 normal) and analyzed by PCR and Sanger sequencing of KLF1 gene.

Results: An already characterized alteration (-148G>A) detected for the first time in the Greek population ( $\beta$ -thalassemia heterozygote, HbF 19,5%) in coexistence with one published variation of unknown significance (c.544T>C, p.Phe182Leu). Two alternations, not yet recorded (g.1980C>A, c.831A>C), were found in  $\beta$ -thalassemia heterozygotes, (HbF: 5,9% and 10,1%) and a frequent polymorphism (c.304T>C, p.Ser102Pro) was present in the majority of tested samples.  $\gamma$ -genes investigation prior to this of KLF1 revealed the polymorphism -158C>T (HBG1, Cretan-type) for the first time in an Albanian origin sample.

Conclusions: KLF1 analysis in a small cohort of samples revealed variations that could possibly contribute in differential HbF expression. More extensive analysis of the above gene along with other non globin genes-modifying factors of HbF expression- has to be conducted especially in  $\beta$ -thalassaemia patients in order to unravel the mechanism of Hb F regulation.

#### P08 Intellectual Disability

##### P08.01

##### The clinical significance of 15q11.2 BP1-BP2 duplications: - Where do we stand?

*S. Serafim<sup>1,2</sup>, B. Marques<sup>1</sup>, S. Pedro<sup>1</sup>, F. Brito<sup>1</sup>, J. Dupont<sup>2</sup>, P. Dias<sup>2</sup>, O. Moldovan<sup>2</sup>, R. Silveira-Santos<sup>2</sup>, S. Custódio<sup>2</sup>, A. Sousa<sup>2</sup>, A. B. Sousa<sup>2</sup>, J. de Sá<sup>3</sup>, A. Queiroz<sup>4</sup>, M. L. Vicente<sup>5</sup>, H. Correia<sup>1</sup>;*

<sup>1</sup>Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal, <sup>2</sup>Hospital de Santa Maria, Centro Hospitalar Lisboa Norte EPE, Lisboa, Portugal, <sup>3</sup>Hospital de Faro E.P.E., Faro, Portugal, <sup>4</sup>Hospital Garcia de Orta, Almada, Portugal, <sup>5</sup>Hospital Amato Lusitano, Castelo Branco, Portugal.

The 15q11.2-q13 region has been well characterized, being associated with a range of syndromic copy number variants (CNVs), and comprises five established break points sites (BP1 to BP5).

While the clinical effect for BP1-BP3, BP2-BP3 and BP4-BP5 CNVs is well established, the same cannot be said for BP1-BP2 CNVs.

Recently the 15q11.2 BP1-BP2 deletion has been reviewed, emerging as a microdeletion syndrome with low penetrance and variable expressivity being the CNV frequently inherited from a healthy parent. This microdeletion is considered to be a risk factor for several neurodevelopment disorders. For the reciprocal duplication the picture has been less conclusive.

Aiming for a better understanding of the clinical significance of this CNV, we collected patients with intellectual disability and/or other clinical features, referred for microarray testing, gathering clinical details for the ones with the duplication. Data was collected from two genetic laboratories.

With a total of 1545 patients, we identified eleven carrying the duplication at 15q11.2 BP1-BP2. It was possible to assess inheritance in only four cases, all inherited from a healthy parent. All patients presented intellectual disability, and facial dysmorphism was the second most common feature observed. Microcephaly, autism, congenital abnormalities, dystonia and cataplexy

where reported individually.

The magnitude of the effect of 15q11.2 duplication remains elusive, and the outcome unclear, posing a major challenge to genetic counseling. Nevertheless, we expect the collection of more of these cases will establish this gain, as it happened with the reciprocal deletion, as a microduplication syndrome with low penetrance and variable expressivity.

#### P08.02

##### Copy number variation at chromosome 16p13.11 in Estonian patients

M. Andresson<sup>1</sup>, P. Tammur<sup>1</sup>, O. Žilina<sup>1</sup>, K. Kuuse<sup>1</sup>, M. Nõukas<sup>2</sup>, T. Reiman<sup>1,3,4</sup>,

<sup>1</sup>Tartu University Hospital, United Laboratories, Department of Genetics, Tartu, Estonia,

<sup>2</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>3</sup>University of Tartu,

Institute of Biomedicine and Translational Medicine, Department of Biomedicine, Tartu, Estonia, <sup>4</sup>University of Tartu, Institute of Clinical Medicine, Department of Pediatrics, Tartu, Estonia.

There are many copy number variations (CNVs) that are associated with susceptibility for neurodevelopmental disorders. One such novel CNV is deletion and duplication at chromosome 16p13.11 whose clinical significance is becoming more ascertained.

CNVs at 16p13.11 show broad phenotypic manifestations and incomplete penetrance. They are significantly enriched in individuals affected by developmental delay/intellectual disability, autism, epilepsy, dysmorphic features and congenital anomalies. Pathogenic 16p13.11 CNVs vary in size, but harbour the critical region called interval II (chr16: 15.48-16.32 Mb, GRCh37/hg19).

We investigated the burden of CNVs at 16p13.11 (HumanCytoSNP-12 v2-1 BeadChips; Illumina Inc.) in a sample of 3,212 individuals with a range of neurodevelopmental conditions, clinically referred for chromosomal microarray analysis. Cases were compared with 14,747 controls from the Estonian Genome Center. We identified 16 patients with CNV within the 16p13.11 region, representing ~ 0.5 % of the patients analyzed, as compared to ~ 0.15 % in the Estonian general population. Eight cases were with deletion and eight with duplication in that region, including one prenatally diagnosed case.

We found that patients with CNV in 16p13.11 present with varied clinical features as previously described. These features are incompletely penetrant. All deletions and duplications identified encompass the critical region of the CNV. The sizes of the rearrangements vary between 0.3-2.7 Mb.

Our findings confirm that genomic abnormalities at chromosome 16p13.11 predispose to a range of neurocognitive and developmental disorders in individuals who carry them

#### P08.03

##### Phenotypic variability associated with the recurrent 1q21.1 copy number variant: eight new cases

A. Perez-Granero<sup>1,2,3</sup>, N. Govea<sup>1,3</sup>, M. Grimalt<sup>1,3</sup>, E. Miravet<sup>1,3</sup>, J. Rosell<sup>1,3,2</sup>,

<sup>1</sup>Hospital Universitari Son Espases, Palma, Spain, <sup>2</sup>Institut de Investigacions Sanitàries de Palma (IDISPA), Palma, Spain, <sup>3</sup>GCV Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

Introduction: The chromosome 1q21.1 locus is a region with a high susceptibility to recurrent deletions and duplications. These disorders have incomplete penetrance and variable expressivity. Hence the clinical significance of this copy-number variation (CNVs) can be difficult to evaluate (from development delay, mild to moderate intellectual disability, autism, psychiatric and behavioural problems to normal phenotype).

Materials and Methods: Our laboratory has examined 664 samples by targeted array comparative genomic hybridization using an (Cytochip ISCATM 8x60 v2.0 Illumina). Samples were received from patients with mental retardation, epilepsy, autism and/or congenital anomalies. When parental samples were available, the familiar study was performed.

Results: We have identified 5 patients with microdeletion and 3 patients with microduplication 1q21.1. One patient with microdeletion was referred for microcephaly and the other 4 patients for developmental abnormalities or mental retardation. In two cases the parents are also affected. We have only one case of parental samples and the deletion was of paternal origin. Two patients with microduplication were referred for epilepsy and the other one by autism. All have macrocephaly. In two cases we have the parental samples and the duplication was of maternal origin in both cases.

Conclusions: In our group of patients the recurrent 1q21.1 CNV is the most frequent anomaly (1,2 %). The macrocephaly is constant in patients with duplication 1q21.1. The family study is inherited origin in all our studied cases. The clinical variability and the incomplete penetrance make genetic counselling very difficult, especially in prenatal diagnosis.

#### P08.04

##### Identifying candidate genes for 2p15p16.1 microdeletion syndrome using clinical, genomic and functional analysis

H. Bagheri<sup>1</sup>, C. Badduke<sup>1</sup>, Y. Qiao<sup>1</sup>, R. Colnaghi<sup>2</sup>, A. Diana<sup>2</sup>, I. Abramowicz<sup>2</sup>, C. Dunham<sup>3</sup>, J. Wen<sup>1</sup>, R. Wildin<sup>4</sup>, M. Nowaczyk<sup>5</sup>, J. Eichmeyer<sup>6</sup>, A. Lehman<sup>3</sup>, B. Maranda<sup>7</sup>, S. Martell<sup>1</sup>, X. Shan<sup>1</sup>, S. Lewis<sup>1</sup>, M. O'Driscoll<sup>8</sup>, C. Gregory-Evans<sup>1</sup>, E. Rajcan-Separovic<sup>1</sup>;

<sup>1</sup>University of British Columbia, Vancouver, BC, Canada, <sup>2</sup>Human DNA Damage Response Disorders Group, Genome Damage & Stability Centre, University of Sussex, Brighton, United Kingdom, <sup>3</sup>BC Children's Hospital, Vancouver, BC, Canada, <sup>4</sup>National Human

Genome Research Institute, Bethesda, Maryland, USA, Bethesda, MD, United States,

<sup>5</sup>Department of Pathology and Molecular Medicine, McMaster University Medical Centre, Hamilton, ON, Canada, <sup>6</sup>St. Luke's Children's Hospital, Boise, ID, United States,

<sup>7</sup>Medical Genetics, Université de Sherbrooke, Sherbrooke, QC, Canada.

Introduction: The 2p15p16.1 microdeletion syndrome has a core phenotype consisting of intellectual disability, microcephaly, hypotonia, delayed growth, common craniofacial features, and digital anomalies. So far, more than 20 cases with this syndrome have been reported in literature; however, the size of the deletions and their breakpoints vary making the identification of candidate genes challenging. Recent reports pointed to four genes (XPO1, USP34, BCL11A and REL) which were included, alone or in combination, in the smallest deletions causing the syndrome.

Results: Herein, we describe 8 new cases with 2p15p16.1 deletion and review all published cases. We demonstrate functional deficits for the above 4 candidate genes using patient lymphoblast cell lines (LCLs) and knockdown of their orthologs in zebrafish. All genes were dosage sensitive based on reduced protein expression in LCLs. In addition, deletion of XPO1, a nuclear exporter, co-segregated with nuclear accumulation of one of its cargo molecules (rpSS5) in patient LCLs. Other pathways associated with these genes (e.g. NF- $\kappa$ B and Wnt signaling as well as DNA damage response) were not impaired in patient LCLs. Knockdown of xpo1a, rel, bcl11aa and bcl11ab resulted in abnormal zebrafish embryonic development including microcephaly, dysmorphic body, hindered growth, small fins as well as structural brain abnormalities.

Conclusions: Our multifaceted analysis strongly implicates XPO1, REL, and BCL11A as candidate genes for the 2p15p16.1 microdeletion syndrome.

#### P08.05

##### Haploinsufficiency of ZNF385B and neurological manifestations in 2q31 microdeletion syndrome

N. Okamoto<sup>1</sup>, K. Shimojima<sup>2</sup>, T. Yamamoto<sup>2</sup>;

<sup>1</sup>Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and, Osaka, Japan, <sup>2</sup>Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan.

Microdeletion of 2q31 involving the HOXD gene cluster is a rare syndrome. The deletion of the HOXD gene cluster is thought to result in skeletal anomalies in these patients. HOX genes encode highly conserved transcription factors that control cell fate and the regional identities along the primary body and limb axes. Several patients with this syndrome have neurological manifestations. We experienced a new patient with 2q31 microdeletion encompassing the HOXD gene cluster and some neighboring genes including the ZNF385B. The patient showed digital anomalies, growth failure, epileptic seizures, and severe intellectual disability. MRI showed delayed myelination and a low signal intensity in the bilateral basal ganglia. Abnormal MRI findings in the 2q31.1 microdeletion syndrome have been reported in some other patients. They were also haploinsufficient for ZNF385B. The ZNF385B is a zinc finger protein expressed in brain. We suggest that haploinsufficiency of ZNF385B is responsible for the neurological features of this syndrome.

#### P08.06

##### 2q37.3 deletions among children with intellectual disability, autism and/or congenital malformations

I. A. Demidova<sup>1,2,3</sup>, S. G. Vorsanova<sup>1,2,3</sup>, K. Vasin<sup>2,1,3</sup>, M. A. Zelenova<sup>2,1,3</sup>, N. S. Shmitova<sup>2</sup>, N. S. Shmitova<sup>2</sup>, O. S. Kurinnaja<sup>1,2,3</sup>, Y. B. Yurov<sup>2,1,3</sup>, I. Y. Iurov<sup>2,1,3</sup>,

<sup>1</sup>Research and Clinical Institute for Pediatrics named after Y.E Veltishev at the Pirogov Russian National Research Medical University, Ministry of Health, Moscow, Russian Federation, <sup>2</sup>Mental Health Research Center, Moscow, Russian Federation, <sup>3</sup>Moscow State University of Psychology and Education, Moscow, Russian Federation.

Introduction. 2q37.3 deletion syndrome is considered to be a rare chromosomal disorder associated with variable clinical features, among which are intellectual disability (ID), autistic features, brachydactyly, short stature, hypotonia and obesity. Some facial features are characteristic for the syndrome (i.e. round face, frontal bossing, arched eyebrows, deep-set eyes, upslanted palpebral fissures, epicanthal folds, thin upper lip, minor ear anomalies). Haploinsufficiency of HDAC4 seems to be the most critical for the phenotypic spectrum. Here, we have attempted to assess chromosome

2q37.3 deletions and HDAC4 haploinsufficiency per se in children with intellectual disability, autism and/or congenital malformation.

Materials and Methods. Molecular karyotyping by SNP/oligonucleotide microarray (resolution: >1kbp) was used to analyze 300 children with ID, autism spectrum disorders and congenital malformations.

Results. Cytogenetically visible 2q37 deletions were detected in 2 cases. Molecular karyotyping confirmed the presence of these deletions (5Mb and 8.5Mb). Furthermore, a CNV within 2q37.3 (44kbp) leading to HDAC4 loss was detected in a patient. All affected female patients had ID, autism and minor ear anomalies. One had widely set nipples and hypotonia. Another one had a short stature. Two patients presented with ocular hypertelorism. HDAC4 haploinsufficiency was observed in all three cases.

Conclusions. We show that 2q37 deletions can be common in children with ID, autism, congenital malformations tending to a frequency as high as 1%. Therefore, one can suggest that 2q37.3 deletions are likely to be more common among these children than previously recognized. Supported by the Russian Science Foundation (Grant #14-35-00060).

#### P08.07

#### 7p21 Interstitial duplication - male patient with global developmental delay, autism, dysmorphisms and epilepsy

J. B. Melo<sup>1,2,3</sup>, S. I. Ferreira<sup>1</sup>, A. Jardim<sup>1</sup>, A. Mascarenhas<sup>4</sup>, M. C. Robalo<sup>4</sup>, I. M. Carreira<sup>1,2,3</sup>,

<sup>1</sup>Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal, <sup>2</sup>CIMAGO – Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal, <sup>3</sup>CNC-IBILI, Universidade de Coimbra, Coimbra, Portugal, <sup>4</sup>Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

Duplications involving the short arm of chromosome 7 are rare events, and most occur in association with rearrangements of other chromosomes as a consequence of missegregation of a balanced translocation in a carrier progenitor. The majority of the patients described have a 7p terminal duplication in association with other imbalance, making 7p interstitial duplications even more rare events. The ones described have variable sizes, usually with cytogenetically visible imbalances, with few characterized by array-Comparative Genomic Hybridization.

Here we report a 12 year old male with global developmental delay, facial dysmorphisms, hypotonia and epilepsy that was analyzed by Agilent 180K oligonucleotide array-CGH, with an average probe spacing of 17Kb. The analysis revealed a 5.5 Mb interstitial duplication in chromosome 7p21. 2p21.1(14,624,128-20187,355)[hg19].

Common to all reported patients with 7p duplications is the presence of intellectual disability and variable facial dysmorphisms, while manifestations like cardiac anomalies, skeletal anomalies and hypotonia are present only in some of the patients. The reported patient is the third reported with autism, after the report of a 7p21.1p22.2 de novo duplication, larger but overlapping the one reported, and a 7p11.2p14.1 proximal duplication. This is the first report of a 7p21 interstitial duplication associated with epilepsy.

Establishing the exact boundaries of 7p pure interstitial duplications, identifying the genes involved and their functional characterization might lead to pinpoint candidate genes responsible for specific manifestations. A detailed clinical characterization of the patients will also help in this genotype-phenotype correlation in order to define critical regions responsible for the different manifestations.

#### P08.08

#### 8p rearrangements detected by array-CGH in a 1500 cohort of patients with intellectual disability

L. M. Pires<sup>1</sup>, S. I. Ferreira<sup>1</sup>, M. Val<sup>1</sup>, N. Lavoura<sup>1</sup>, J. Saraiva<sup>2</sup>, I. M. Carreira<sup>1,3,4</sup>, J. B. Melo<sup>1,3,4</sup>,

<sup>1</sup>Laboratório de Citogenética e Genómica, Faculdade de Medicina, Coimbra, Portugal,

<sup>2</sup>Serviço de Genética Médica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, <sup>3</sup>CIMAGO – Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal, <sup>4</sup>CNC-IBILI, Universidade de Coimbra, Coimbra, Portugal.

Array-Comparative Genomic Hybridization (array-CGH) has increased the diagnostic yield in patients with intellectual disability (ID), autism spectrum disorders (ASD) and multiple congenital anomalies due to its improved resolution. The short arm of chromosome 8 (8p) is structurally complex because of the existence of two olfactory gene clusters flanking a 5Mb region of 8p23.1, several low copy repeats (LCRs) and common inversion polymorphisms that makes this region prone to various recurrent genomic rearrangements that include: 8p23.1 deletions or duplications, 8p23.1 paracentric inversions, pericentric inversions, 8p translocations, 8p inverted duplication with associated terminal deletion, among others. In addition to these 8p pathogenic imbalances, others have been reported as copy number variants (CNVs) without apparent clinical significance. In our cohort of 1500 patients,

with ID and/or ASD studied by Agilent 180K oligonucleotide array-CGH, we have detected 14 patients with 8p imbalances (5 deletions, 8 duplications and one inverted duplication). Single gene deletions were observed in 3 patients and larger interstitial deletions in 2 patients (one with a 3,4Mb 8p21.2 deletion and the other with two adjacent deletions in 8p23.1 and in 8p23.1p21.3. Interstitial duplications were identified in 8 patients, including a 6,3Mb 8p21.3p21.1 duplication, a 960Kb 8p23.1 duplication, a 274Kb 8p22 duplication, 4 patients with a small 160Kb duplication inherited from healthy parents classified as a CNV of unknown clinical significance and one patient with a single gene duplication. In brief, our results are in accordance with published data suggesting existence of multiple hotspots in 8p causing complex rearrangements and variety of phenotypes.

#### P08.09

#### ADNP mutations are a frequent cause of autosomal dominant intellectual disability

A. Riess<sup>1</sup>, U. Moog<sup>1</sup>, S. Waldmüller<sup>1</sup>, P. Bauer<sup>1</sup>, O. Riess<sup>1</sup>, S. Karch<sup>2</sup>, E. Schröck<sup>4</sup>, N. Di Donato<sup>4</sup>, A. Rump<sup>4</sup>, L. Mackenroth<sup>4</sup>, A. Tzschach<sup>4</sup>,

<sup>1</sup>Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Tübingen, Germany, <sup>2</sup>Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany, <sup>3</sup>Center for Child and Adolescent Medicine, Heidelberg University Hospital, Heidelberg, Germany, <sup>4</sup>Technische Universität Dresden, Institute of Clinical Genetics, Dresden, Germany.

Heterozygous loss-of-function mutations in the activity dependent neuro-protector homeobox gene ADNP, which encodes a transcription factor involved in the SWI/SNF remodelling complex, were recently shown to be associated with moderate to severe intellectual disability (ID). Additional clinical abnormalities which included short stature, muscular hypotonia and facial dysmorphisms had enabled the delineation of a new entity termed Helsmoortel-van der Aa syndrome (OMIM 615873). Since its first report in 2014, studies in large ID cohorts identified mutations in 14 unrelated patients, suggesting that ADNP mutations are a comparatively frequent cause of autosomal dominant ID.

We report on three unrelated German patients (two boys and one girl) in whom ADNP mutations were detected by next-generation sequencing. Clinical problems that are in accordance with those of previously reported patients included severe ID, muscular hypotonia, short stature and hypoplastic corpus callosum. Borderline microcephaly, which had so far only been reported in a minority of patients, was present in two of our patients. One patient had unilateral iris coloboma, which has hitherto not been reported in association with ADNP mutations.

Our findings corroborate the assumption that ADNP mutations are one of the more frequent causes of ID in sporadic patients, and they expand the clinical spectrum of the probably underdiagnosed Helsmoortel-van der Aa syndrome.

#### P08.10

#### Clinical and quantitative PCR confirmation of copy number variations detected by array CGH

A. C. Ceylan<sup>1</sup>, E. Z. Taşkiran<sup>1</sup>, P. Ö. Şimşek Kiper<sup>2</sup>, G. E. Utine<sup>2</sup>, K. Boduroğlu<sup>2</sup>, M. Alikaşifoğlu<sup>1,2</sup>,

<sup>1</sup>Hacettepe University, Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Hacettepe University, Faculty of Medicine, Department of Pediatrics, Department of Pediatric Genetics, Ankara, Turkey.

Introduction: Copy Number Variations (CNV) are known to underlie several diseases such as intellectual disability, autism spectrum disorders, and schizophrenia. It is also well known that CNVs play important roles in microdeletion syndromes. Several methods were developed to detect CNVs. One of these methods, array Comparative Genomic Hybridization (aCGH), is now the recommended first-tier test for molecular diagnosis in patients with cognitive disorders. aCGH detects genome-wide CNVs, however, findings should be validated. Thus, the aim of this study was to verify CNVs detected by aCGH in patients with cognitive disorders.

Materials and methods: aCGH (Agilent® 8x60K) analyses for 150 patients with cognitive disorders detected 19 CNVs - either of unknown clinical significance or of likely pathogenicity. Afterwards, these CNVs were verified by SYBR Green-based quantitative-PCR (qPCR).

Results: Verification was done in 18 CNVs. Of these, 4 CNVs turned out to be false positives. Two CNVs were de novo. Clinical correlation of the CNVs revealed that these 16 CNVs were among the already known microdeletion and/or microduplication syndromes. In order to draw attention to the importance of verification, two patients will be presented: One with a confirmed and paternally inherited deletion at 2q37, and the other with a false positive duplication at 1q21.3.

Conclusion: The current study has shown that CNVs detected by aCGH

should always be verified, for example by qPCR, especially when there is an impact on clinical phenotype. Verification is also important for the family for accurate genetic counseling.

#### P08.11

#### Implementation of arrayCGH in a Portuguese tertiary hospital: 5 years and over 1400 cases

**R. Silveira-Santos, A. Sousa, S. Custódio, S. Serafim, J. Dupont, O. Moldovan, P. Dias, A. Medeira, I. Cordeiro, A. B. Sousa;**  
Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria - CHLN, Lisboa, Portugal.

Successful application of arrayCGH to prenatal and postnatal genetic diagnosis led to a significant increase in the detection rate of chromosomal abnormalities. Here, we reflect on our 5-year experience (2011 to 2015) with the implementation of arrayCGH.

Different array platforms were used with increasing probe density and, since 2013, included SNPs to investigate patients whose phenotype was consistent with an imprinting disorder or an autosomal recessive disorder caused by autozygosity. We examined 1414 patients belonging to 1261 families (95 prenatally, 1319 postnatally), who were referred mostly for intellectual disability, autism and/or congenital abnormalities.

In 10/95 (11%) prenatal and in 365/1319 (28%) postnatal cases we identified possibly relevant copy number variations (CNV) or regions of absence of heterozygosity (AOH). Overall there were 1125 relevant CNVs that required further investigation originating 468 reportable CNVs (203 deletions, 265 duplications), ranging in size from 43kb (intragenic deletion of *MBD5*) to 154Mb (X aneuploidy). After segregation analysis and/or confirmatory tests, 266 variants were classified as pathogenic and 202 as of uncertain clinical significance. We also detected an AOH containing a gene associated with Bardet-Biedl syndrome, consistent with the patient's phenotype, and a case of partial UPD(14).

Large size rearrangements can be benign and, conversely, very small size CNVs may be pathogenic. The global analysis of our array database suggests a postnatal cut-off of 30kb and, for prenatal reporting, a 150kb cut-off for deletions and a 200kb for duplications. However, we recommend analyzing each case on an individual basis and in close connection with the clinicians.

#### P08.12

#### ARV1 - a new candidate gene for intellectual disability and seizures

**R. Segev<sup>1,2</sup>, A. Aran<sup>1,2</sup>, H. Fridman<sup>1</sup>, P. Renbaum<sup>1</sup>, S. Gulsuner<sup>3</sup>, S. Zeligson<sup>1</sup>, T. Walsh<sup>3</sup>, M. King<sup>3</sup>, E. Levy-Lahad<sup>1,2</sup>;**

<sup>1</sup>Shaare Zedek Medical Center, Jerusalem, Israel, <sup>2</sup>Hadassah Medical School, the Hebrew University, Jerusalem, Israel, <sup>3</sup>Departments of Medical Genetics and Genome Sciences, University of Washington, Seattle, WA, United States.

Intellectual disability (ID) and seizures are genetically heterogeneous. We report two male siblings with severe ID and seizures, born at term to consanguineous Palestinian parents. Both have non-verbal communication and severe ID without regression, seizures started in the first year of life. Hearing and vision are normal. Both are normocephalic, brain MRI demonstrated mild cortical atrophy in the older brother only. Lactate, ammonia, Acyl-carnitine, liver function and lipid profile were normal.

Whole Exome sequencing of the affected siblings, both parents and two unaffected sisters revealed only one variant that was homozygous in both affected children, heterozygous in the sisters: *ARV1* p.G189R (c.G565A). This residue is highly conserved in all vertebrates, GERP score of 4.7. This variant has not been reported in 1000 Genome or EVS. Only 2 heterozygotes reported in ExAC (frequency e-051.648), predicted as damaging by Polyphen (0.998), and likely pathogenic in Clinvar, based on a single indication of this variant in a family with an unspecified neurodegenerative disease (Alazami 2015).

*ARV1* is an endoplasmic reticulum transmembrane protein, first identified as essential for sterol esterification in yeast. *Arv1*-/- mice exhibit a dramatic lean phenotype. In mammalian cells, *Arv1* is required for normal cholesterol, bile acid and triglyceride homeostasis, its knockdown increases lipid-mediated cell death. The effects of the G189R mutation on these functions will be presented.

Our results suggest *ARV1* mutations cause functional brain abnormalities, should be considered as a candidate gene for ID with seizures, and provide further evidence of the role of sterol metabolism in neurological function.

#### P08.13

#### Clinical utility of whole exome sequencing: case report of a child with global neurodevelopmental delay focused on autism spectrum disorder

**A. Vallmajó<sup>1</sup>, D. Casellas<sup>1</sup>, A. Castan<sup>1</sup>, M. García<sup>1</sup>, B. Rodríguez-Santiago<sup>2</sup>, M. Obón<sup>1</sup>;**  
<sup>1</sup>Hospital U de Girona Dr. Josep Trueta, Girona, Spain, <sup>2</sup>qGenomics Laboratory, Barcelona, Spain.

Introduction: Autism Spectrum Disorders (ASD, OMIM#209850) are a group of heterogeneous neurobehavioral syndromes without an accurate molecular genetics protocol after a non-conclusive karyotype, fragile-X and CGH-array tests results. Here we report the case of a two-years-old child, born at 41 weeks gestation after induced labor for oligohydramnios, with a global neurodevelopmental delay focused on ASD, a suggestive hereditary family history and normal karyotype, fragile-X and CGH-array tests. Whole exome sequencing was performed to depict the complex genetics autism spectrum disorder.

Material and methods: Exome sequencing and selective analysis of ASD known genes was applied at first. Then a second analysis extended to remaining OMIM genes was carried out (qGenomics Laboratory)

Results: ASD related genes analysis did not reveal any significant finding, but the extended study to other OMIM genes identified a likely pathogenic mutation in *GDI1* gene (c.389-1G>C; hemizygous splicing variant). This gene has been strongly associated with intellectual disabilities (ID) although there is only evidence from 3 published families with X-linked ID and *GDI1* mutations (one nonsense, two missense) (Strobl-Wildemann G, et al. Am J Med Genet A. 2011).

Conclusions: The mutation found in the studied family provides more evidence about the *GDI1* role in X-linked ID. Furthermore, *GDI1* seems to be related also to another clinical feature, ASD. Our findings suggest including *GDI1* in ASD panels as well as in X-linked intellectual disabilities. Finally, our work underlines the utility of whole exome sequencing instead of specific gene panels for an accurate diagnosis and complete genetic counselling.

#### P08.14

#### Targeted gene panel to investigate intellectual disability and autism spectrum disorders comorbidity

**E. Leonardi, A. Gasparini, M. Carraro, E. Bettella, R. Polli, F. Cesca, S. Sartori, S. C. E. Tosatto, A. Murgia;**  
University of Padua, Padova, Italy.

Intellectual disability (ID) and autism spectrum disorder (ASD) are clinically and genetically heterogeneous diseases. Recent whole exome sequencing studies indicated that genes associated with different neurologic disorders are shared among these disorders or converge on common functional pathways.

The aim of this study was to design a customized multiplex PCR-based gene panel to investigate the genetic bases of ID and ASD comorbidity. The gene panel covers 74 genes, including a subset of genes that are found recurrently mutated in ID or ASD conditions, genes shared among ID and ASD disease network and ID/ASD genes that are directly connected to these. We analyzed 18 clinically selected patients with ID and ASD without syndromic features, negative for Fragile-X test and array CGH. For all 18 patients, 85-97% of the targeted regions achieved read depths of at least 20x, with average read depths ranging from 91x to 612x. 22 rare single nucleotide variants (SNV) were found in the exonic sequences of 16 out of the 18 patients: most of them are predicted to be deleterious and have very low frequency or are not present in public databases. Although familial segregation analysis will be helpful to determine the pathogenic nature of the variants, likely causative variants have been assigned for 8 (44%) of the patients. These results confirm the diagnostic value of the targeted gene panel for investigating children with ID and ASD.

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#### P08.15

#### Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability

**G. R. Monroe<sup>1</sup>, G. W. Frederix<sup>2</sup>, S. M. C. Savelberg<sup>1</sup>, T. I. de Vries<sup>1</sup>, K. J. Duran<sup>1</sup>, J. J. van der Smagt<sup>1</sup>, P. A. Terhal<sup>1</sup>, P. M. van Hasselt<sup>3</sup>, H. Y. Kroes<sup>1</sup>, N. M. Verhoeven-Duijf<sup>1</sup>, I. J. Nijman<sup>1</sup>, E. C. Carbo<sup>1</sup>, K. L. van Gassen<sup>1</sup>, N. V. Knoers<sup>1</sup>, A. M. Hövels<sup>2</sup>, M. M. van Haelst<sup>1</sup>, G. Visser<sup>2</sup>, G. van Haften<sup>1</sup>;**

<sup>1</sup>Utrecht University Medical Center, Utrecht, Netherlands, <sup>2</sup>Utrecht Institute of Pharmaceutical Sciences, Utrecht, Netherlands, <sup>3</sup>Wilhelmina Children's Hospital, Utrecht, Netherlands.

Introduction: This study investigated whole-exome sequencing (WES) yield in a subset of intellectually disabled patients referred to our clinical diagno-

stic center and calculated the total costs of these patients' diagnostic trajectory in order to evaluate early WES implementation.

**Materials and Methods:** We compared 17 patients' trio-WES yield with the retrospective costs of diagnostic procedures by comprehensively examining patient records and collecting resource use information for each patient, beginning with patient admittance and concluding with WES initiation. We calculated cost savings using scenario analyses to evaluate the costs replaced by WES when used as a first diagnostic tool.

**Results:** WES resulted in diagnostically useful outcomes in 29.4% of patients. The entire traditional diagnostic trajectory average cost was \$16,409 per patient, substantially higher than the \$3,972 trio-WES cost. WES resulted in average cost savings of \$3,547 for genetic and metabolic investigations in diagnosed patients and \$1,727 for genetic investigations in undiagnosed patients.

**Conclusion:** The increased causal variant detection yield by WES and the relatively high costs of the entire traditional diagnostic trajectory suggest that early implementation of WES is a relevant and cost efficient option in patient diagnostics. This information is crucial for centers considering implementation of WES and serves as input for future value-based research into diagnostics.

#### P08.16

#### A 1.77 Mb deletion in 3p26.3 encompassing CNTN6 and CNTN4 genes: case report

F. Brito<sup>1</sup>, B. Marques<sup>1</sup>, S. Pedro<sup>1</sup>, S. Serafim<sup>1</sup>, R. Goncalves<sup>2</sup>, J. Freixo<sup>2</sup>, H. Correia<sup>1</sup>;

<sup>1</sup>Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal, <sup>2</sup>Hospital D. Estefânia, Centro Hospitalar de Lisboa Central EPE, Lisboa, Portugal.

Chromosome microarray analysis is a powerful diagnostic tool and is being used as a first-line approach to detect chromosome imbalances associated with intellectual disability, dysmorphic features and congenital abnormalities. This test enables the identification of new copy number variants (CNVs) and their association with new microdeletion/microduplication syndromes in patients previously without diagnosis. We report the case of a 7 year-old female with moderate intellectual disability, severe speech delay and auto and hetero aggressivity with a previous 45,XX,der(13;14)mat karyotype performed at a younger age. Affymetrix CytoScan 750K chromosome microarray analysis was performed detecting a 1.77 Mb deletion at 3p26.3, encompassing 2 OMIM genes, *CNTN6* and *CNTN4*. These genes play an important role in the formation, maintenance, and plasticity of functional neuronal networks. Deletions or mutations in *CNTN4* gene have been implicated in intellectual disability and learning disabilities. Disruptions or deletions in the *CNTN6* gene have been associated with development delay and other neurodevelopmental disorders. The haploinsufficiency of these genes has been suggested to participate to the typical clinical features of 3p deletion syndrome. Nevertheless inheritance from a healthy parent has been reported, suggesting incomplete penetrance and variable phenotype for this CNV. We compare our patient with other similar reported cases, adding additional value to the phenotype-genotype correlation of deletions in this region.

#### P08.17

#### Modeling of chromosomal diseases related to intellectual disability using genome editing of induced pluripotent stem cells from a patient with CNTN6 gene microdeletion

T. A. Shnayder<sup>1</sup>, A. G. Menzorov<sup>1,2</sup>, N. M. Matveeva<sup>1</sup>, V. S. Fishman<sup>1</sup>, I. E. Pristyazhnyuk<sup>1</sup>, T. V. Karamysheva<sup>1</sup>, A. A. Kashevarova<sup>3,4</sup>, N. A. Skryabin<sup>3,4</sup>, E. A. Sazhenova<sup>3</sup>, I. N. Lebedev<sup>3,4,5</sup>, O. L. Serov<sup>1,2</sup>;

<sup>1</sup>Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation, <sup>2</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>3</sup>Institute of Medical Genetics, Tomsk, Russian Federation, <sup>4</sup>National Research Tomsk State University, Tomsk, Russian Federation, <sup>5</sup>Siberian State Medical University, Tomsk, Russian Federation.

**Introduction:** Previously we have described three patients with intellectual disability and some dysmorphic features. Patients had microdeletion or microduplication at 3p26.3, affecting a single gene, *CNTN6* (Kashevarova et al., 2014). In *Cntn6* knock-out mice lack of this gene causes abnormal dendritogenesis. We decided to produce patient-specific neurons to study molecular mechanisms of *CNTN6* deletion effect in humans.

**Materials and Methods:** Induced pluripotent stem cells (iPSCs) were produced from patient skin fibroblasts with 3p26.3 microdeletion and healthy donor using standard protocol with four transcription factors. Array-based comparative genomic hybridization (aCGH), RT-PCR and FISH analyses were used to confirm microdeletion in iPSCs. CRISPR/Cas9 system was used to knock-out *CNTN6* allele. Neuronal differentiation of iPSCs was performed by NGN2 overexpression (Zhang et al., 2013).

**Results:** Eight iPSC lines had diploid karyotype (46,XY). Pluripotency was

assessed by generation of embryoid bodies and teratoma formation in SCID mice. We confirmed microdeletion and lack of *de novo* chromosome aberrations by aCGH. In addition, we produced targeted missense mutation in the remaining intact *CNTN6* allele using CRISPR/Cas9 system to "enhance" effects of *CNTN6* deletion. iPSCs neuronal differentiation was directed by overexpression of transcription factor NGN2. According to preliminary data, nearly all cells were positive for neuron specific markers and had electrophysiological parameters comparable to those of a healthy donor.

**Conclusions:** Patient-specific iPSC-derived neurons with *CNTN6* deletion and complete knock-out could be used as a unique model for studying effects caused by *CNTN6* copy number changes.

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#### P08.18

#### Eight further individuals with intellectual disability and epilepsy carrying biallelic CNTNAP2 aberrations allow delineation of the mutational and phenotypic spectrum

M. Smogavec<sup>1</sup>, A. Smith<sup>2</sup>, J. Hoyer<sup>3</sup>, D. Lederer<sup>4</sup>, M. Nassogne<sup>5</sup>, E. E. Palmer<sup>6,7</sup>, M. Deprez<sup>4</sup>, V. Benoit<sup>4</sup>, I. Maystadt<sup>4</sup>, C. Noakes<sup>2</sup>, A. Leaf<sup>3</sup>, A. Reis<sup>3</sup>, D. Shears<sup>8</sup>, K. Brockmann<sup>9</sup>, C. Zweier<sup>3</sup>;

<sup>1</sup>Institute of Human Genetics, University Medical Center, Georg August University, Göttingen, Germany, <sup>2</sup>Oxford Medical Genetics Laboratories, Churchill Hospital, Oxford, United Kingdom, <sup>3</sup>Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, <sup>4</sup>Centre de Génétique Humaine, Institut de Pathologie et Génétique, Charleroi (Gosselies), Belgium, <sup>5</sup>Pediatric Neurology Unit, Saint-Luc University Hospital, UCL, Brussels, Belgium, <sup>6</sup>GOLD (Genetics of Learning and Disability) Service, Hunter Genetics, Waratah, Australia, <sup>7</sup>University of New South Wales, Sydney, Australia, <sup>8</sup>Department of Clinical Genetics, Churchill Hospital, Oxford University Hospitals NHS Trust, Oxford, United Kingdom, <sup>9</sup>Interdisciplinary Pediatric Center for Children with Developmental Disabilities and Severe Chronic Disorders, University Medical Center, Georg August University, Göttingen, Germany.

**Introduction:** Heterozygous copy number variants (CNVs) or sequence variants in the contactin associated protein 2 gene CNTNAP2 have been discussed as risk factors for a wide spectrum of neurodevelopmental and neuropsychiatric disorders. Biallelic aberrations in this gene are causative for an autosomal-recessive disorder with epilepsy, severe intellectual disability (ID) and cortical dysplasia (CDFES), however, due to the limited number of reported individuals, the full mutational and clinical spectrum has still to be characterized.

**Methods and Results:** Targeted sequencing, chromosomal microarray analysis or multi gene panel sequencing identified homozygous mutations, compound heterozygous CNVs or compound heterozygous CNVs and mutations in eight individuals from six unrelated families. All aberrations were inherited from healthy, heterozygous parents and are predicted to be deleterious for protein function. Epilepsy occurred in all patients with onset in the first three and a half years of life. Further common aspects were severe ID (7/8), regression of speech development (5/8), and behavioural anomalies (7/8). Interestingly, cognitive impairment in one of two affected brothers was in comparison relatively mild with good speech and simple writing abilities. Cortical dysplasia that was previously reported in CDFES, was not present in MRIs of six individuals and only suspected in one.

**Conclusion:** By identifying novel homozygous or compound heterozygous, deleterious CNVs and mutations in CNTNAP2 in eight individuals from six independent families with moderate to severe ID, early onset epilepsy and behavioural anomalies, we considerably broaden the mutational and clinical spectrum associated with biallelic aberrations in CNTNAP2.

#### P08.19

#### Coffin-Lowry syndrome, a familial "female only" case; natural history and variability of Coffin-Lowry syndrome in females

A. Kattentidt<sup>1</sup>, R. Kersseboom<sup>1</sup>, T. Rinne<sup>2</sup>, E. van der Zon<sup>1</sup>;

<sup>1</sup>Zuidwester, Spijkenisse, Netherlands, <sup>2</sup>Department of Clinical Genetics, Radboud University Medical Centre, Nijmegen, Netherlands.

Coffin-Lowry syndrome (CLS) is a rare X linked semi-dominant mental retardation syndrome, in males characterized by severe intellectual disability, growth retardation, distinct facial features and progressive kyphoscoliosis. In females, the phenotype is less explicit and ranges from the full phenotype as seen in males, to completely absent. The diagnosis in females can be challenging. Furthermore, data on the natural history in affected females is still scarce.

We describe a familial case of CLS with two affected adult females and no affected males. The molecular analysis of the *RPS6KA3* gene revealed a heterozygous frameshift mutation in exon 11, once previously reported in the literature. Both sisters presented well-known features of CLS: intellectual

disability, facial dysmorphic features and soft, fleshy hands. Both females were affected by a psychiatric disorder, including psychotic disorder and depression. Interestingly, there was a strongly intrafamilial variability in the severity of psychiatric disease and psychosocial functioning. Both parents were asymptomatic. The genetic testing to determine whether the mother is an asymptomatic carrier is ongoing.

To our knowledge this is one of the very few families with affected females only. This case demonstrates the variability of clinical phenotype and natural history of CLS in adult females. Furthermore, we illustrate the challenges of treatment of a psychiatric disorder in females with CLS.

akattentidt@gmail.com

#### P08.20

#### Clinical, chromosomal and molecular characterization of a cohort of 160 patients with corpus callosum agenesis with or without intellectual disability

C. Depienne<sup>1,2,3</sup>, C. Nava<sup>1,2</sup>, A. Rastetter<sup>1</sup>, S. Heide<sup>1,2</sup>, C. Mignot<sup>2,4</sup>, B. Keren<sup>2</sup>, F. Lesne<sup>2</sup>, A. Faudet<sup>2</sup>, L. Boutaud<sup>5</sup>, C. Alby<sup>5</sup>, C. Bole<sup>5</sup>, P. Nitchké<sup>6</sup>, T. Billette de Villemeur<sup>6</sup>, M. Moutard<sup>6</sup>, T. Attié-Bitach<sup>2</sup>, D. Héron<sup>2,4</sup>;

<sup>1</sup> Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, Paris, France, <sup>2</sup> Département de Génétique, Hôpital Pitié-Salpêtrière, APHP, Paris, France, <sup>3</sup> Service de cytogénétique, Hôpitaux universitaires de Strasbourg, Strasbourg, France, <sup>4</sup> Centre de Référence Déficiences Intellectuelles de Causes Rares, Hôpital Pitié-Salpêtrière, APHP, Paris, France, <sup>5</sup> Institut Imagine, Hôpital Necker, APHP, Paris, France, <sup>6</sup> Service de Neuropédiatrie, Hôpital Trousseau, APHP, Paris, France.

Corpus callosum agenesis (CCA) corresponds to complete or partial absence of the corpus callosum, the main brain structure connecting the two hemispheres, and is the most frequent brain malformation in humans. CCA is usually diagnosed antenatally, and prenatal counseling is therefore a crucial issue, especially in apparently isolated CCA. To determine the type and frequency of genetic anomalies and to identify new genes involved in CCA, we recruited prospectively a cohort of 150 patients with CCA and intellectual disability (ID), and several families with CCA without ID. Clinical examination has allowed establishing a diagnosis for 10 patients, whereas a chromosomal abnormality was identified in 20 patients. Next, we have sequenced 423 candidate genes selected for their known or putative association with CCA in humans (345) or mouse models (79) in 100 probands. A pathogenic mutation was identified in 18 patients in 8 different genes (ARID1B, ZBTB18, TUBA1A, FOXG1, SPTAN1, ARX, MED12, DYNC1H1). In addition, we have sequenced the exome of 5 trios, 4 sib pairs, and 3 families with CCA without ID segregating as a dominantly inherited trait. Our results show that next generation sequencing approaches are powerful diagnostic tools for CCA and confirm the large genetic heterogeneity of this condition, in which many genes are still unknown. Moreover, the identification of genes involved in CCA without ID will change the paradigm of prenatal genetic counseling.

#### P08.21

#### Customized high resolution arrayCGH for neurodevelopmental disorders: our experience

M. Rincic<sup>1</sup>, Z. Krsnik<sup>1</sup>, K. Gotovac<sup>2</sup>, T. Liehr<sup>3</sup>, F. Borovecki<sup>2</sup>, L. Brecevic<sup>1</sup>;

<sup>1</sup> Croatian Institute for Brain Research, School of Medicine University of Zagreb, Zagreb, Croatia, <sup>2</sup> Department for Functional Genomics, University Hospital Centre Zagreb, University of Zagreb School of Medicine, Centre for Translational and Clinical Research, Zagreb, Croatia, <sup>3</sup> Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany.

Array comparative genomic hybridization (aCGH) is a powerful tool for detecting relative small genomic imbalances. Here we report on the design and potential of a targeted aCGH for the evaluation of patients with neurodevelopmental disorders. An array was designed on Agilent's SureDesign web application in 8X60K format, covering 6,803 genes, with a probe spacing of 0.66-20 kb.

Overall 39 patients were analyzed and 479 genes had aberrant copy number (CNV). These genes were screened by an algorithm for brain specific gene expression (publicly available data). Expression heat maps were found for 316 genes: 93 were below the expression threshold of 5.5, 134 had expression level up to 9, and 86 had high expression level up to 13, respectively. Comparing custom array with commercially available Agilent 8X60K platforms, the custom one is superior in terms of numbers of specific genes covered. Furthermore, comparing the resolution of arrays (33 kb SurePrint 3G, 60 kb ISCA), our design has much higher average resolution of 8.2 kb and therefore has a capability to detect smaller CNVs. Considering that our custom array was able to detect imbalances in 220 genes that are expressed in brain, the design presented showed to be a powerful tool for detecting

genomic imbalances in genes that are involved in neurodevelopmental disorders. This preliminary data are strong indications that implementation of targeted custom aCGH in research may reveal new candidate genes underlying neurodevelopmental disorders.

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#### P08.22

#### Epilepsy and its correlation to psychomotor development degree in the Spanish cohort of Wolf-Hirschhorn syndrome

J. Nevado<sup>1,2</sup>, R. Blanco<sup>3</sup>, I. Málaga<sup>3</sup>, J. J. Granizo<sup>4</sup>, P. Barruz<sup>1</sup>, P. D. Lapunzina<sup>1</sup>;

<sup>1</sup> INGEMM, Hospital Universitario la Paz-IdiPaz, MADRID, Spain, <sup>2</sup> CIBERER, Madrid, Spain, <sup>3</sup> Hospital Central de Asturias, Oviedo, Spain, <sup>4</sup> Hospital Infanta Cristina, MADRID, Spain.

Wolf-Hirschhorn syndrome (WHS) is a contiguous gene syndrome caused mainly by deletion of the short arm of chromosome 4. Epilepsy represents a major clinical challenge during early years in WHS, with significant impact on quality of life. The main aim of this work it will be to describe social, demographic and clinical features of a cohort of WHS in Spain trying to establish whether or not the level of psychomotor development in this cohort correlates with size of the deletion and other clinical features.

**Methods:** Using high-resolution SNP-array, we finely mapped CNVs in around 40 individuals with WHS. Seizure and other phenotypes data were collected through a comprehensive questionnaire supplemented with available medical records, and statically analyzed.

**Results:** We show a complete description of a cohort of WHS in our country. Although main clinical features were similar to other previous studies, our cohort showed a better degree of psychomotor development than other ones. In fact, seizures in this cohort were preferentially under a febrile context (91%), and related to communication skills; more than 60% was able to communicate (non-verbal), 46% say words, and 27% to make short sentences, in a context that positively correlates with shorter deletions.

**Conclusions:** We founded a direct correlation between size of the deletion, severity of the epilepsy and the level of psychomotor development delay. Our preliminary results suggested that epilepsy in WHS may be a complex event and may it involve the synergistic effects of loss of two or more genes.

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#### P08.24

#### Exome trio analysis as an effective approach in deciphering the genetic etiology of rare diseases

M. Peña-Vilabenda<sup>1</sup>, J. Botet<sup>1</sup>, P. Maietta<sup>1</sup>, M. Martínez-García<sup>1</sup>, A. Fernández-Jaén<sup>2</sup>, D. Martín Fdez.-Mayoralas<sup>2</sup>, A. L. Fernández<sup>2</sup>, A. Patiño<sup>2</sup>, I. Espejo Portero<sup>4</sup>, M. Alonso Cerezo<sup>5</sup>, A. Mesas Arostegui<sup>6</sup>, A. Santana Rodríguez<sup>7</sup>, I. Málaga<sup>8</sup>, J. Solera Santos<sup>9</sup>, M. A. Motos Guirao<sup>10</sup>, B. Gil Fournier<sup>11</sup>, L. Martorell Sampol<sup>12</sup>, M. D. Miramar Gallart<sup>13</sup>, J. Bernar Solano<sup>14</sup>, S. Alvarez<sup>1</sup>;

<sup>1</sup> NGS Laboratory, NIMGenetics, Madrid, Spain, <sup>2</sup> Hospital Universitario Quirón, Madrid, Spain, <sup>3</sup> Clínica Universidad de Navarra, Pamplona, Spain, <sup>4</sup> Hospital Universitario Reina Sofía, Córdoba, Spain, <sup>5</sup> Hospital Universitario de la Princesa, Madrid, Spain, <sup>6</sup> Hospital El Ángel, Málaga, Spain, <sup>7</sup> Hospital Universitario Materno Infantil de Canarias, Las Palmas, Spain, <sup>8</sup> Instituto Neuroológico Dr. Mateos, Oviedo, Spain, <sup>9</sup> Complejo Hospitalario Universitario de Albacete, Albacete, Spain, <sup>10</sup> Centro de Estudios Genéticos de Andalucía, Granada, Spain, <sup>11</sup> Hospital Universitario de Getafe, Madrid, Spain, <sup>12</sup> Hospital Sant Joan de Déu, Barcelona, Spain, <sup>13</sup> Hospital Miguel Servet, Zaragoza, Spain, <sup>14</sup> Hospital Ruber Internacional, Madrid, Spain.

**Introduction:** Whole exome sequencing (WES) has entered the medical practice as an effective diagnostic test transforming the molecular diagnosis and clinical management of undiagnosed genetic diseases. Exome trio analysis is a very effective strategy to identify de novo, hemizygous, newly homozygous and in compound heterozygous potentially causal variants of rare genetic disorders.

**Methods:** We performed exome sequencing using Ion AmpliSeqTM Exome RDY technology (Life Technologies) with Ion ProtonTM and Ion S5-XLTM. Sequencing reads were analysed using Torrent Suite software. Trio annotated variants using ION Reporter were prioritized with an in-house analytical pipeline to identify causative genetic variants.

**Results:** We present the analysis of the first 130 trios referred to NIMGenetics. Patients were mainly children with syndromic intellectual disability (55%). The genetic etiology was potentially elucidated in 55 probands harboring 38 causal variants and 22 likely causative variants, achieving a 43% molecular diagnostic rate. Among these patients, 36 harbored de novo variants, 6 hemizygous maternally inherited variants, 4 in compound heterozygous variants, 7 newly homozygous variants and 3 variants inherited from parents. Patients with syndromic intellectual disability (54%, 38/70) and specific neurological disorders (53%, 9/17) showed higher molecular diagnostics rates than patients with non-neurologic disorders (27%, 3/11).

and non-syndromic intellectual disability (19%, 6/32).

Conclusions: In our cohort exome trio analysis provide a diagnostic yield of 43% in patients whom traditional molecular diagnostics strategies were uninformative. The implementation of WES as a first-tier diagnostic approach will provide a higher diagnostic yield and a cost-efficient option particularly in rare syndromic intellectually disabled patients.

#### P08.25

#### Offspring of an FMR1 premutation carrier: son with a deletion of FMR1 exon 1 and daughter with a full mutation

**A. Baumer, E. Wey, R. Abbas, S. M. Azzarello-Burri, A. Rauch;**  
Institute of Medical Genetics, Schlieren, Switzerland.

The vast majority (approx. 99%) of fragile X syndrome patients present with a trinucleotide repeat expansion (>200 CGG repeats) in the 5' UTR of the FMR1 gene. Deletions and point mutations are detected in less than 1% of patients. Deletions affecting single exons or the entire FMR1 gene arise either de novo, are transmitted from mothers carriers of the same deletion, or derive from a maternal pre- or full-mutation allele.

We report on the transmission of a mutant FMR1 allele from a premutation carrier mother to her two children. The healthy mother is carrier of an FMR1 allele with an expansion in the premutation range (approximately 84 CGG repeats). A deletion of the entire exon 1 was detected in her 2-year-old son diagnosed with fragile X syndrome. The analyses were based on a RP PCR and a methylation sensitive MLPA assay, including CNV analysis. A prenatal investigation for her daughter showed that she is carrier of a full mutation with approximately 285 CGG repeats. This latter analysis was performed on chorionic villi using RP PCR. The results for both siblings showed no evidence for mosaicism.

This case report draws attention to the fact that premutations may not only result in repeat expansions but also in deletions affecting the FMR1 gene.

#### P08.26

#### New insight into genotype-phenotype correlation in patients with FOXG1 point mutation

**D. Mitter<sup>1</sup>, M. Pringsheim<sup>2</sup>, M. Kaulisch<sup>3</sup>, R. E. Lutz<sup>4</sup>, B. Conover<sup>4</sup>, N. S. Hauser<sup>5</sup>, T. A. Grebe<sup>6</sup>, C. Courage<sup>7</sup>, B. Zoll<sup>8</sup>, W. Heinritz<sup>9</sup>, R. Westman<sup>10</sup>, L. Rohena<sup>11</sup>, K. Ramsey<sup>12</sup>, J. Cohen<sup>13</sup>, S. A. Fatemi<sup>13</sup>, N. Sakkubai<sup>13</sup>, S. Schröder<sup>14</sup>, K. Plümacher<sup>14</sup>, G. J. Kluger<sup>14</sup>, J. Lemke<sup>15</sup>, K. L. Helbig<sup>15</sup>, I. Preh<sup>16</sup>, K. Hörtnagel<sup>16</sup>, B. Zirn<sup>17</sup>, K. Brockmann<sup>14</sup>,**  
<sup>1</sup>Institute of Human Genetics, Leipzig, Germany, <sup>2</sup>Klinik für Neurologiatrie und Neurologische Rehabilitation, Epilepsiezentrum, Schön Klinik, Vogtareuth, Germany, <sup>3</sup>, Leipzig, Germany, <sup>4</sup>Department of Genetic Medicine, University of Nebraska Medical Center, Omaha, NE, United States, <sup>5</sup>Department of Genetic Medicine and Metabolism, Children's Hospital Central California, Madera, CA, United States, <sup>6</sup>Division of Genetics and Metabolism, Phoenix Children's Hospital, Phoenix, AZ, United States, <sup>7</sup>Division of Human Genetics, University Children's Hospital Inselspital, Bern, Switzerland, <sup>8</sup>Institut für Humangenetik, Universitätsmedizin, Göttingen, Germany, <sup>9</sup>Praxis für Humangenetik, Cottbus, Germany, <sup>10</sup>Children's Specialty Center, St. Luke's Children's Hospital, Boise, ID, United States, <sup>11</sup>Medical Genetics, San Antonio Military Medical Center, San Antonio, TX, United States, <sup>12</sup>Center For Rare Disorders, Phoenix, AZ, United States, <sup>13</sup>Moser Center for Leukodystrophies and Neurogenetics Service, Kennedy Krieger Institute, Johns Hopkins Medical Institution, Baltimore, MD, United States, <sup>14</sup>Klinik für Kinder- und Jugendmedizin Universitätsmedizin, Göttingen, Germany, <sup>15</sup>Division of Clinical Genomics, Ambry Genetics, Aliso Viejo, CA, United States, <sup>16</sup>CeGaT, Tübingen, Germany, <sup>17</sup>Genetikum Stuttgart, Genetic counselling and diagnostics, Stuttgart, Germany.

The FOXG1 syndrome is a rare autosomal dominant developmental encephalopathy. In the literature, 53 patients with a point mutation in the FOXG1 gene have been reported (until December 2015). Through international collaboration, we collected data from 30 new patients with a heterozygous FOXG1 point mutation, including 18 novel mutations, and present detailed phenotypic description and neuroimaging. We performed systematic statistical analysis and genotype-phenotype correlation in patients with FOXG1 syndrome based on the molecular and clinical data of a total of 83 patients with a point mutation in FOXG1. Our analysis shows a higher phenotypic variability than expected. The most characteristic clinical features in patients with a FOXG1 mutation are: (1) severe microcephaly, including primary microcephaly, (2) short stature, (3) moderate to severe psychomotor delay, with some patients achieving unsupported walking and speech, (4) preservation of social interaction, (5) frequent neurological abnormalities including hypotonia, stereotypies, dyskinesia, strabismus, bruxism, and spasticity, (6) variable forms of epilepsy, (7) abnormal sleep pattern, (8) gastrointestinal features, (9) development of scoliosis or kyphoscoliosis, and (10) brain anomalies. Our results show a correlation between location and coding effect of the FOXG1 mutation. The most severe phenotype is associated with truncating mutations in the 5' domain and the fork head domain, the mildest phenotype is associated with missense mutations in the fork head conserved site 1. These data serve for improved interpretation of new FOXG1 sequence variants and well-founded genetic counselling.

#### P08.27

#### Foxp1-related intellectual disability syndrome: a recognizable entity

**I. Meerschaert<sup>1,2</sup>, J. Pêtre<sup>3</sup>, N. Revencu<sup>3</sup>, N. Bockaert<sup>2</sup>, A. Oostra<sup>2</sup>, O. Vanakker<sup>1,2</sup>, M. Vilenov<sup>4</sup>, T. J. L. de Ravel<sup>5</sup>, D. Mekahli<sup>5</sup>, K. K. Vaux<sup>6</sup>, J. Sebat<sup>7</sup>, F. F. Hamdan<sup>8</sup>, J. L. Michaud<sup>9</sup>, P. Lapunzina<sup>9</sup>, N. Di Donato<sup>10</sup>, L. Hudgins<sup>11</sup>, A. K. Hanson-Kahn<sup>11</sup>, B. Dallapiccola<sup>12</sup>, A. Novelli<sup>12</sup>, J. Andrieux<sup>13</sup>, J. Morton<sup>14</sup>, N. Ragge<sup>14</sup>, J. Radley<sup>14</sup>, M. J. Parker<sup>15</sup>, K. Neas<sup>16</sup>, A. Dheedene<sup>1</sup>, B. Menten<sup>1</sup>, D. Lederer<sup>17</sup>, B. Callewaert<sup>1,2</sup>,**

<sup>1</sup>Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium,

<sup>2</sup>Department of Pediatrics, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Centre Génétique, Cliniques Universitaires Saint-Luc, Brussels, Belgium, <sup>4</sup>NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States, <sup>5</sup>Centre for Human Genetics, University Hospital Leuven, Leuven, Belgium, <sup>6</sup>Gleeson Laboratory for Neurodevelopmental Genetics, University of California, San Diego, CA, United States,

<sup>7</sup>Beyster Center for Genomics of Psychiatric Diseases, University of California, San Diego, CA, United States, <sup>8</sup>CHU Saint Justine Research Center, Montréal, QC, Canada, <sup>9</sup>Instituto de Genética Médica Y Molecular, Hospital Universitario La Paz, Madrid, Spain, <sup>10</sup>Institut für Klinische Genetik, Technische Universität Dresden, Dresden, Germany, <sup>11</sup>Department of Genetics, Stanford University School of Medicine, Stanford, CA, United States,

<sup>12</sup>Laboratorio Di Genetica Medica, Ospedale Pediatrico del Bambino Gesu', Rome, Italy,

<sup>13</sup>Institut de Génétique Médicale, Hospital Jeanne de Flandre, Lille, France, <sup>14</sup>Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, United Kingdom, <sup>15</sup>Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield, United Kingdom,

<sup>16</sup>Genetic Health Service NZ, Wellington, New Zealand, <sup>17</sup>Centre de Génétique Humaine, Institut de Pathologie et de Génétique asbl, Gosselies, Belgium.

**Introduction:** Intellectual disability (ID) and specific language impairment (SLI) cover a wide range of conditions with heterogeneous phenotypes and etiologies, making them challenging for clinical geneticists. Mutations in forkhead box protein P1 (FOXP1), a transcription regulator, are associated with ID and SLI, with or without autistic features (MIM: 613670). Multiple FOXP1 patients have been described, though so far, no specific phenotype emerged.

**Materials and Methods:** We report on the clinical and molecular data of 21 novel and well-characterized patients with FOXP1 mutations and review the 25 previously reported FOXP1 patients to delineate the condition.

**Results:** Phenotypic analysis shows a combination of neuromotor delay, ID, SLI, and typical facial features including a high broad forehead, bent downslanting palpebral fissures, ptosis, blepharophimosis, and a short nose with bulbous tip. Relative macrocephaly, strabismus, a wide mouth with down-turned corners, and pronounced nasolabial folds appear more variable. Behavioural problems and autistic features are common. Brain, cardiac and urogenital malformations seem associated. Molecular data show *de novo* mutations in all cases, either monogenic or part of a more extended interstitial 3p deletions. Subgroup analysis reveals sensorineural hearing loss and more severe ID in patients with interstitial 3p deletions.

**Conclusions:** FOXP1-related ID syndrome is a recognizable entity that seems more frequent than expected. We expand the phenotypic description with typical facial features and associated organ system involvements. This will enable clinicians to make a gestalt diagnosis and will be helpful to evaluate genotype-phenotype correlations when interpreting large NGS data obtained in patients with ID and/or SLI.

#### P08.28

#### Identification of de novo FOXP1 variants in three unrelated individuals.

**L. I. Alba Valdivia<sup>1</sup>, S. García Miñáur<sup>2,3,4</sup>, P. Lapunzina Badía<sup>1,3,4</sup>, E. Vallespín<sup>1,3,4</sup>, L. Fernández<sup>1,3,4</sup>, Á. del Pozo Mate<sup>6,1,3,4</sup>, K. Ibáñez Garikano<sup>1,3,4</sup>, J. Silla<sup>1,3,4</sup>, F. Santos-Simarro<sup>1,3,4</sup>, M. Mori<sup>1,3,4</sup>, R. Mená<sup>1,3,4</sup>, V. Fernández Montaño<sup>1</sup>, P. Tirado Requero<sup>5</sup>, E. Mansilla<sup>1,3,4</sup>, J. Nevado<sup>1,3,4</sup>, M. de Torres<sup>1,3,4</sup>, M. Palomares Bralo<sup>1,3,4</sup>,**

<sup>1</sup>Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>INGEMM. Hospital Universitario La Paz, Madrid, Spain, <sup>3</sup>Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain,

<sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, Madrid, Spain, <sup>5</sup>Neurología Infantil, Hospital Universitario La Paz, Madrid, Spain.

**Background and aims:** FOXP1 is a member of the Forhead Box P (FOXP) subfamily of transcription factors, which also includes FOXP2. Disruptions of FOXP2, the closest relative to FOXP1, have been involved in the etiology of familial verbal dyspraxia.

In the last few years, 11 individuals with FOXP1 variants have been reported, supporting the implication of this gene in the pathogenesis of human cognitive disorders including language impairment. These patients show autism spectrum disorders, intellectual disability, global developmental delay and moderate to severe speech delay.

We report on three new individuals with de novo FOXP1 variants showing features that are consistent with this new clinical entity (MIM 613670).

**Method:** Whole genome aCGH was carried out in patients 1 and 2 using a custom-designed 60K oligonucleotide array (KaryoArray®v3.0).

Whole exome sequencing was performed as a trio in patient 3 to identify de novo mutations related to the phenotype after a normal result using aCGH.

**Results:** Patient 1 had a 379 Kb de novo deletion that only affected FOXP1. Patient 2 carried a 6.28 Mb de novo deletion including FOXP1 and other ge-

nes. In patient 3 a de novo nonsense mutation (p.R525X) was identified. Discussion and conclusions:

These cases contribute to the characterization of the emerging phenotype associated with protein-disrupting FOXP1 variants.

Screening for FOXP1 variants should be considered when evaluating individuals with developmental delay, intellectual disability, autism and severe language defects.

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### P08.31

#### Pathogenic long indels identified in patients with intellectual disability

*S. Pajusala<sup>1,2</sup>, R. Pfundt<sup>3</sup>, L. E. L. M. Vissers<sup>3</sup>, M. P. Kwint<sup>3</sup>, T. Reimand<sup>1,2,4</sup>, K. Öunap<sup>1,4</sup>, J. A. Veltman<sup>3,5</sup>, J. Y. Hehir-Kwa<sup>3</sup>,*

<sup>1</sup>Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia, <sup>2</sup>Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, <sup>3</sup>Department of Human Genetics, Donders Institute, Radboudumc, Nijmegen, Netherlands, <sup>4</sup>Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia, <sup>5</sup>Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, Netherlands.

Whole exome sequencing (WES) continues to facilitate the genetic diagnosis of patients with intellectual disability (ID). Sensitive methods exist for the identification of single nucleotide variants (SNVs) and indels less than 20bp in size. However, the discovery of long indels 20 - 200bp in size, remains challenging even in non-repetitive regions of the genome such as exons. As a result the role of small deletions (long indels) is currently unknown and under reported in many studies.

We analysed the WES data from 98 patients with ID to identify pathogenic long indels located in exons of 650 ID genes using Pindel and Platypus. All patients had previously screened negative for WES based SNV, small indel and large copy number variants. To assess the sensitivity of the calling algorithms we compared variants discovered in the patient cohort to 35 exonic indels described in the Genome of the Netherlands dataset with an allele frequency greater than 5%, 20-200bp in size.

We were able to detect 74% (n=26) of common exonic indels, serving as an estimated detection sensitivity. Further analysis of the rare variants within the patient cohort identified two indels predicted to explain the patients' clinical syndrome (diagnostic yield 2%). A 42bp homozygous deletion of exon-intron border in PGAP3 gene, and a 114bp heterozygous complex indel disrupting the MECP2 gene.

Despite the fragmented nature and short read length of WES data, using specific software tools for long indel detection increases diagnostic yield in patients with ID.

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### P08.32

#### De novo 16p13.3 duplication in a girl with intellectual disability, behavioural abnormality and dysmorphic features - characterization of 16p13.3 duplication without CREBBP gene

*D. Gieruszczak-Biatek<sup>1,2</sup>, A. Skorka<sup>1,2</sup>, M. Kucharczyk<sup>2</sup>, M. Mlynek<sup>2</sup>, M. Kugaudo<sup>1,2</sup>,*

<sup>1</sup>The Medical University of Warsaw, Warsaw, Poland, <sup>2</sup>The Childrens Memorial Health Institute, Warsaw, Poland.

The availability and use of CGH with high-resolution microarrays has greatly improved the detection of micro-deletion and duplications in patients with developmental delay and congenital anomalies. Here we report the molecular karyotyping and phenotypic description of a new patient with 16p13.3 duplication. Our patient presented with intellectual disability, behavioural abnormality and dysmorphic features. Whole-genome oligonucleotide microarray (PerkinElmer CGXTM HD 4x180K Oligo Array) analysis revealed a 2.41 Mb duplication on chromosome 16p13.3 (chr16:788,264-3,203,164; hg19) encompassing 124 genes (including 71 OMIM genes) as well as the critical regions for alpha thalassemia with intellectual disability (AT-ID), tuberous sclerosis 2 (TSC2), and polycystic kidney disease 1 (PKD1). FISH studies in both parents gave normal results, proving the de novo occurrence of this aberration in the child. According to the literature, 16p13.3 duplication consists of a contiguous gene syndrome with variable phenotypic expression, and CREBBP is the critical gene responsible for the main clinical features of 16p13.3 microduplication. To date, there is no evidence of other genes in this region contributing to the phenotype. It is suggested that other genes play additive or modulator role justifying some of the features found in individuals with dup 16p13.3. In the presented girl, the 16p13.3 duplication is responsible for the clinical phenotype, although it does not include the CREBBP gene. We indicate another possible candidate genes, contributing additional information for this microduplication syndrome and providing new data supporting further genotype-phenotype studies.

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### P08.33

#### PACS2 de novo missense mutation in two patients presenting with intellectual disability and recognizable facial dysmorphism

*N. Jean-Marçais<sup>1,2</sup>, K. Tattan-Brown<sup>3</sup>, D. Héron<sup>4</sup>, E. Backer<sup>5</sup>, The Deciphering Developmental Disorders Study, J. Donadieu<sup>6</sup>, J. Rivière<sup>2,7</sup>, D. Lehalle<sup>1,2,7</sup>, A. Bruel<sup>7</sup>, Y. Dufourd<sup>2,7</sup>, J. Thevenon<sup>1,2,7</sup>, L. Faivre<sup>1,2,7</sup>, C. Thauvin<sup>1,2,7</sup>,*

<sup>1</sup>Centre de Génétique, Centre Hospitalier Universitaire Dijon, F-21079 Dijon, France,

<sup>2</sup>Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (TRANSLAD), Centre Hospitalier Universitaire Dijon, F-21079 Dijon, France,

<sup>3</sup>South West Thames regional Genetics Service University, St George's university, NHS Foundation Trust, London, United Kingdom, <sup>4</sup>Service de Génétique, Hôpital Pitié-Salpêtrière, Assistance Publique des Hôpitaux de Paris, Paris, France, <sup>5</sup>Genomic Diagnostics Laboratory, Manchester Centre for Genomic Medicine, Central Manchester University Hospitals, NHS Foundation Trust, Saint Mary's Hospital, Manchester, United Kingdom, <sup>6</sup>Department of Internal Medicine & French Reference Center for Rare Auto-immune & Systemic Diseases, Hôpital Pitié-Salpêtrière, Assistance Publique des Hôpitaux de Paris, Paris, France, <sup>7</sup>Equipe d'Accueil 4271, Génétique des Anomalies du Développement, Université de Bourgogne, F-21079 Dijon, France.

Syndromic or non-syndromic intellectual disability (ID) has multiple underlying etiologies including in particular monogenic rare disease. Most of these causes are currently unknown. In the last five years, the development of whole exome sequencing (WES) has lead to identify many new genes responsible of ID. Using WES we identified the same de novo missense heterozygous mutation (p.Glu209Lys) in the PACS2 gene in two patients presenting with moderate ID, neonatal seizures and similar recognizable facial dysmorphism (bulbous nasal tip, wide mouth, thin upper lip and everted lower lip). A moderate neutropenia is observed in one of them. This mutation involves a highly conserved amino acid in an acid domain constituted by ramified amino acids, leading to possible polarity and protein conformation changes. The PACS2 gene encodes for a protein mainly expressed in brain, heart, kidney, pancreas and testis. PACS2 is involved in controlling the exchanges between the endoplasmic reticulum (ER) and mitochondria, in ER homeostasis and in apoptosis induction. Cellular studies have demonstrated that PACS2 knockout would lead to ER dissociation and mitochondria fragmentation, as well as a defect in tBid-mediated apoptosis. PACS2 is also a paralog to PACS1 (54% of identical sequence) previously reported in a human disorder characterized by ID. These data allow considering PACS2 as a new gene responsible for ID with recognizable facial features. The identification of other individuals will be the next step for the description of this new syndrome.

### P08.34

#### Characterisation of emerging pathological CNVs in adults with intellectual disabilities and co-morbid psychiatric disorders

*K. Wolfe, A. Strydom, A. McQuillin, N. Bass;*

*University College London, London, United Kingdom.*

Chromosomal copy number variations (CNVs) are highly implicated in the aetiology of neurodevelopmental disorders. Research in pediatric cohorts with developmental delay/intellectual disabilities (ID) has led to the identification of new CNV syndromes. Many adults with ID harboring rare CNV syndromes remain unidentified, precluding description of later onset psychiatric phenotypes.

In an attempt to further characterise very rare emerging CNV syndromes we undertook genome wide chromosomal microarray analysis and comprehensive psychiatric phenotyping of adults with idiopathic ID recruited from ID psychiatry services across England.

Of the 202 adults recruited to the study 11% had CNVs classed as likely pathogenic by the clinical diagnostic laboratory. These included a 1.7Mb deletion at 2q13 and a 2.4Mb duplication at 4p16.3, both of which are poorly characterised in adulthood. We undertook further targeted recruitment of children and adults with CNVs in these regions to expand the phenotypic data on these emerging pathological CNVs.

Study of adults with idiopathic ID and psychiatric disorders has enabled the phenotypic characterisation of two CNVs associated with co-morbid pathologies. Ongoing characterisation of rare CNVs in adulthood could inform clinical management of children with emerging CNV syndromes.

**P08.35****Six individuals with intellectual disability, cerebral white matter abnormalities and motor impairment are homozygous for a splice site mutation in the ABCC9 gene**

G. Å. M. Hansen<sup>1</sup>, B. Nygård<sup>1</sup>, H. Hjellnes<sup>1</sup>, R. Glad<sup>1</sup>, M. Falkenberg Smedland<sup>1</sup>, V. Marton<sup>1</sup>, C. Jonsrød<sup>1</sup>, M. Van Gheluwe<sup>1,2</sup>;

<sup>1</sup>Department of Medical Genetics, Division of Child and Adolescent Health, University Hospital of North Norway, Tromsø, Norway, <sup>2</sup>Department of Clinical Medicine, Faculty of Health Sciences, The Arctic University of Tromsø, Tromsø, Norway.

Two families with members displaying substantial motor impairment, mild intellectual disability and periventricular white matter abnormalities on cerebral MRI were referred to our department. Four affected siblings in family 1 had been thoroughly investigated for neuromuscular disorders, including mitochondrial disease. Two siblings in family 2 were previously diagnosed with tuberous sclerosis due to cerebral MRI abnormalities. A trio from each family was analysed using the Trusight One sequencing panel, targeting 4813 disease associated genes on an Illumina MiSeq platform. All six patients (10-31 years old) were homozygous for a splice site mutation c.1320+1G>A in ABCC9. ABCC9 encodes sulfonylurea receptor 2 (SUR2), which is a regulatory subunit of the K-ATP-sensitive potassium (KATP) channel. These SUR2-containing KATP channels are enriched in the sarcolemma, where they sense intracellular ADP content and trigger the opening or closing of potassium channels. These channels are critical in cells during periods of high energy demand. The outcome ABCC9 of the c.1320+1G>A variant is an in frame deletion (r.1165\_1320del) resulting (if translated) in a SUR2 protein lacking 52 amino acids (p.Ala389\_Gln440del). This compromised SUR2 protein might explain some of the clinical findings in these patients. Whereas heterozygous mutations in ABCC9 are known to cause Cantu syndrome (hypertrichotic osteochondrodysplasia, OMIM#239850), we speculate if the homozygous mutation identified in these two families may cause a new, distinct syndrome.

**P08.36****Haploinsufficiency of histone acetylase modifier BRPF1 is responsible for mild intellectual disability with ptosis and might participate to clinical manifestations of the 3p deletion syndrome**

F. Mattioli<sup>1</sup>, E. Schaefer<sup>2</sup>, K. Yan<sup>3</sup>, A. Magee<sup>4</sup>, G. Vieville<sup>5</sup>, K. Dieterich<sup>5</sup>, C. Philippe<sup>6</sup>, X. Yang<sup>7</sup>, J. Mandel<sup>1</sup>, A. Piton<sup>1</sup>;

<sup>1</sup>IGBMC, Strasbourg, France, <sup>2</sup>Service de génétique médicale, Strasbourg, France,

<sup>3</sup>Goodman Cancer Center & Department of Medicine McGill University, Montreal, QC, Canada, <sup>4</sup>Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast, Ireland, <sup>5</sup>Hôpital Couple – Enfant, Grenoble, France, <sup>6</sup>Medical Genetics Service, Sainte-Justine Hospital, Montréal, QC, Canada, <sup>7</sup>Goodman Cancer Center & Department of Medicine McG, Montréal, QC, Canada.

Intellectual disability (ID) is a common neurodevelopmental disorder characterized by an extreme genetic heterogeneity with more than five hundred genes now described as implicated in mendelian forms. We performed exome sequencing in a large family with five affected individuals presenting with mild ID, ptosis, growth retardation and hypotonia with a suspected autosomal dominant mode of inheritance. We identified a 2-bp deletion causing a frameshift in BRPF1, a gene not yet implicated in ID. The mutated transcript is expressed in fibroblasts and leads to a truncated protein. The BRPF1 protein activates two histone acetyltransferases (KAT6A and KAT6B) causing syndromic ID in human when mutated. We have therefore investigated how the truncation can affect BRPF1 interaction with its partners KAT6A and KAT6B, as well as its histone H3 binding ability. BRPF1 is located in the 3p25 region, involved in a deletion syndrome characterized by ID with additional features, and presumably due to the loss of the SETD5 gene. The identification of a truncating mutation in the BRPF1 gene perfectly cosegregating with ID in one large family with phenotype overlapping the 3p25 deletion syndrome suggests that BRPF1 might also participate to the cognitive and dysmorphic phenotype of this syndrome, and especially ptosis, hypotonia, and growth retardation, as these clinical features are present in patients with 3p25 deletion only when BRPF1 is included. In addition, we also report few other patients with similar phenotype carrying BRPF1 deletions or point mutations, confirming the involvement of the gene in syndromic mild ID.

**P08.37****Clinical and functional characterization of patients with *de novo* mutations and deletions of YY1**

A. T. Vulto-van Silfhout<sup>1</sup>, M. Gabriele<sup>2</sup>, R. Kumar<sup>3</sup>, J. H. M. Schuurs-Hoeijmakers<sup>1</sup>, L. E. L. M. Vissers<sup>1</sup>, W. M. Nillesen<sup>1</sup>, E. Douglas<sup>4</sup>, S. Kjaergaard<sup>5</sup>, A. Stray-Pedersen<sup>6</sup>, S. A. Lynch<sup>7</sup>, E. Frengen<sup>8</sup>, D. Rodriguez-Buriticá<sup>9</sup>, C. Schaaf<sup>10</sup>, J. Friedman<sup>11</sup>, P. L. Germann<sup>2</sup>, A. Vitriolo<sup>2</sup>, J. A. Rosenfeld<sup>10</sup>, K. Kosaki<sup>12</sup>, A. Rauch<sup>13</sup>, J. Gecz<sup>2</sup>, G. Testa<sup>2</sup>, B. B. A. de Vries<sup>1</sup>, D. A. Koolen<sup>1</sup>,

<sup>1</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen,

Netherlands, <sup>2</sup>Department of Experimental Oncology, Laboratory of Stem Cell Epigenetics, European Institute of Oncology, Milan, Italy, <sup>3</sup>School of Medicine and the Robinson Research Institute, The University of Adelaide, Adelaide, Australia, <sup>4</sup>Genetics and Molecular Pathology, SA Pathology, North Adelaide, Australia, <sup>5</sup>Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark, <sup>6</sup>Human and Medical Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>7</sup>National Centre for Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland, <sup>8</sup>Department of Medical Genetics, University of Oslo and Oslo University Hospital, Oslo, Norway, <sup>9</sup>Division of Genetics, Department of Pediatrics, University of Texas Health, Houston, TX, United States, <sup>10</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>11</sup>Departments of Neurosciences and Pediatrics, UCSD/Rady Children's Hospital, San Diego, CA, United States, <sup>12</sup>Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan, <sup>13</sup>Institute of Medical Genetics, University of Zurich, Schlieren-Zurich, Switzerland.

Trio-based exome sequencing of 10 patients with intellectual disability (ID) previously identified a *de novo* missense mutation in YY1<sup>1</sup>, a transcription factor with an important role in various biological processes, such as proliferation, differentiation, embryogenesis, apoptosis, and tumor development. Our study aimed to determine the role of YY1 mutations in ID. Additional patients with *de novo* mutations in YY1 were collected through targeted resequencing of YY1, further exome sequencing studies, and through Gene-Matcher<sup>2</sup> and

Decipher<sup>3</sup>. In total, eight patients with *de novo* mutations in YY1 (four missense and four truncating mutations) and three patients with small deletions encompassing YY1 were identified. All patients showed below average IQ that ranged from moderate ID to mild learning problems. Other recurrently observed features included intrauterine growth retardation, feeding problems, behavioral problems, and dystonia. In four patients, typical facial dysmorphisms were present: facial asymmetry with a broad forehead, fullness of the upper eyelids, and a Gingko leaf-like shape of the upper lip. To gain insight into the effects of YY1 mutations on downstream signaling, we profiled lymphoblastoid cell lines from patients with mutations and deletions of YY1 through RNA-seq and ChIP-seq for YY1 and enhancer chromatin marks. In conclusion, we show that *de novo* mutations in the transcription factor YY1 are recurrently observed in patients with ID, and we present a characterization of the main gene network nodes affected by YY1 mutations and deletions.

References<sup>1</sup>Vissers et al, 2010, Nat Genet<sup>2</sup>Sobreira et al, 2015, Hum Mutat<sup>3</sup>Chatzimichali et al, 2015, Hum Mutat

**P08.38****Identification of new candidate genes in intellectual disability and epilepsy**

J. Halvardson<sup>1</sup>, J. Zhao<sup>1</sup>, A. Zaghlool<sup>1</sup>, C. Wentzel<sup>1</sup>, P. Georgii-Hemming<sup>2</sup>, E. Måansson<sup>3</sup>, H. Ederth Sävmark<sup>4</sup>, G. Brandberg<sup>5</sup>, C. Soussi Zander<sup>1</sup>, A. Thuresson<sup>1</sup>, L. Feuk<sup>1</sup>;

<sup>1</sup>Uppsala University, Uppsala, Sweden, <sup>2</sup>Karolinska Institute, Stockholm, Sweden, <sup>3</sup>Örebro University Hospital, Örebro, Sweden, <sup>4</sup>Gävle Hospital, Gävle, Sweden, <sup>5</sup>Falun Pediatric Clinic, Falun, Sweden.

It has been estimated that at least 20-30% of intellectual disability (ID) patients are co-diagnosed with epilepsy, representing a strong over-representation of epilepsy in ID patients compared with the general population prevalence of 0.5-1 %. Here we present the results of exome sequencing in 39 trio families where the index patient is diagnosed with both ID and epilepsy. The families have no history of disease and have previously been screened for copy number variation using arrays.

In 39 patient-parent trios we identified 29 *de novo* mutations in coding sequence. Analysis of *de novo* and inherited variants using standard guidelines for interpretation of coding variants yielded a molecular diagnosis in 11 families (28.2%). Variants in genes of unknown clinical significance were further investigated using protein and co-expression networks, as well as previously published exome sequencing results in neurodevelopmental disorders. Both network analysis and a number of previously published *de novo* mutations implicate the gene HECW2 as a novel candidate gene in ID and epilepsy.

Our results also highlight the utility of network analysis and comparison to previous large-scale studies as a way to prioritize candidate genes for further studies. This study adds to the increasingly growing list of causative and candidate genes in ID and epilepsy and highlights HECW2 as a new candidate gene in neurodevelopmental disorders.

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## P08.39

### The French HUGODIMS consortium experience on intellectual disabilities

*S. Bézieau<sup>1</sup>, S. Kury<sup>1</sup>, T. Besnard<sup>1</sup>, X. Latypova<sup>1</sup>, A. Denommé<sup>2</sup>, B. Cogné<sup>1</sup>, S. Schmitt<sup>1</sup>, P. Boisseau<sup>1</sup>, M. Vincent<sup>3</sup>, S. Mercier<sup>1</sup>, B. Isidor<sup>4</sup>, HUGODIMS;*

<sup>1</sup>CHU de Nantes, Nantes, France, <sup>2</sup>CHU d'Angers, Angers, France.

**Background:** Intellectual disabilities (ID) constitute a heterogeneous group of syndromic and non-syndromic disorders of variable prevalence. The number of genes accounting for the vast majority of ID is so important that a targeted analysis is questionable. Numerous studies pointed to the relevancy of exome sequencing of patients/parents trios to increase diagnostic yield.

**Purpose:** Our goal was to determine the efficiency of exome sequencing to unravel the molecular cause of ID in patients with severe phenotype seen by clinical geneticists from Western France hospitals.

**Method:** Following a trio-based exome sequencing strategy, we investigated 75 patients with severe ID which could not be explained by fragile X syndrome, copy number variations (CGH array) or even by known candidate genes.

**Results:** For almost 50% of the cases tested so far, we have been able to explain the molecular cause of the disease or highlight new ID candidate genes.

**Conclusions:** Our work confirmed that the trio-based whole-exome sequencing is a powerful approach for diagnosis and research. New identified genes highlighted the importance of several signaling pathways such as the NMDAR one, in the occurrence of neurodevelopmental disorders.

## P08.40

### MED13L haploinsufficiency syndrome - four additional cases emphasizing the variability of the phenotype

*P. M. Tørring<sup>1</sup>, L. B. Ousager<sup>1</sup>, C. Brasch-Andersen<sup>1</sup>, M. J. Larsen<sup>1</sup>, L. N. Krogh<sup>1</sup>, U. Dunkhase-Hein<sup>2</sup>, M. Kibæk<sup>3</sup>, L. Laulund<sup>3</sup>, N. Illum<sup>3</sup>, M. Zollino<sup>4</sup>, C. R. Fagerberg<sup>5</sup>;*

<sup>1</sup>Dept. of Clinical Genetics, Odense University Hospital, Odense C, Denmark, <sup>2</sup>Dept. of Pediatrics, Hospital of Southern Jutland, Aabenraa, Denmark, <sup>3</sup>Dept. of Pediatrics, Odense University Hospital, Odense C, Denmark, <sup>4</sup>Istituto di Genetica Medica, Università Cattolica Sacro Cuore, Rome, Italy.

**Introduction:** *MED13L* haploinsufficiency syndrome has recently been described and is characterized by moderate intellectual disability (ID), speech impairment, and dysmorphic facial features, in some cases accompanied by congenital heart defects. We present four additional patients with *MED13L* haploinsufficiency syndrome and review the literature for phenotypical and genetic aspects of previously described patients.

**Materials and methods:** In the search for genetic aberrations in individuals with ID, two of the patients were identified by chromosomal microarray analysis, and one by exome sequencing. One of the individuals, suspected of *MED13L* haploinsufficiency syndrome, based on clinical features, was identified by sanger sequencing of *MED13L*.

**Results:** All four patients had *de novo* *MED13L* aberrations, including two intragenic *MED13L* microdeletions, one nonsense and one frameshift *MED13L* mutation. Phenotypically, they all had ID, speech and motor delay, open mouth appearance, and macrostomia. Two patients were diagnosed with autism, and three had macroglossia. None had congenital heart defects.

The literature was reviewed with respect to clinical features and genetic aberrations.

**Conclusions:** Even if most clinical features of *MED13L* haploinsufficiency syndrome is rather non-specific, the syndrome may be suspected in patients with developmental delay, speech impairment, and macroglossia, macrostomia, or open mouth appearance.

## P08.41

### Dominant mutations in the splicing factor PUF60 cause a recognizable syndrome with intellectual disability, heart defects and short stature

*S. El Chehadeh<sup>1,2</sup>, W. S. Kerstjens-Frederikse<sup>3</sup>, J. Thevenon<sup>2,4</sup>, P. Kuentz<sup>4,2,5</sup>, A. Bruel<sup>4</sup>, C. Thauvin-Robinet<sup>2,4</sup>, C. Bensignor<sup>6</sup>, H. Dollfus<sup>1,7</sup>, V. Laugel<sup>7,8</sup>, J. Riviere<sup>2,4,5</sup>, Y. Duffourd<sup>2</sup>, C. Bonnet<sup>9</sup>, M. Robert<sup>10</sup>, R. Isaico<sup>11</sup>, M. Straub<sup>11</sup>, C. Creuzot-Garcher<sup>11</sup>, P. Calvas<sup>12</sup>, N. Chassaing<sup>12</sup>, C. Gilissen<sup>12</sup>, R. Pfundt<sup>13</sup>, J. S. Klein Wassink-Ruiter<sup>3</sup>, L. Faivre<sup>2,4</sup>,*

<sup>1</sup>Service de génétique médicale, Institut de génétique médicale d'Alsace (IGMA), Centre de Référence Maladies Rares «Anomalies du développement et syndromes malformatifs» de l'Est, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France, <sup>2</sup>FHU TRANSLAD, Centre de Référence Maladies Rares «Anomalies du développement et syndromes malformatifs» de l'Est, Centre de Génétique, CHU de Dijon, Dijon, France, <sup>3</sup>Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands, <sup>4</sup>EA 4271, Génétique et Anomalies du Développement (GAD), Université de Bourgogne, Dijon, France, <sup>5</sup>Laboratoire de Biologie Moléculaire, Plateau technique de biologie, CHU de Dijon, Dijon, France, <sup>6</sup>Service de Pédiatrie, Hôpital d'enfants, CHU de Dijon, Dijon, France, <sup>7</sup>U1112 Laboratoire de Génétique Médicale, Faculté de médecine, Université de Strasbourg, Strasbourg,

France, <sup>8</sup>Service de Neuropédiatrie, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France, <sup>9</sup>Service de Cardiopédiatrie, Hôpital d'enfants, CHU de Dijon, Dijon, France, <sup>10</sup>Service d'Ophtalmologie, APHP, Hôpital Necker Enfants Malades, Paris, France, <sup>11</sup>Service d'Ophtalmologie, Hôpital civil, CHU de Dijon, Dijon, France, <sup>12</sup>Service de Génétique Médicale, Hôpital de Purpan, CHU de Toulouse, Toulouse, France, <sup>13</sup>Department of Human Genetics, Radboud Institute for Molecular Life Sciences and Donders Centre for Neuroscience, Radboud University Medical Center, Nijmegen, Netherlands.

The PUF60 gene encodes for the Poly-U Binding Splicing Factor 60 kDa which belongs to the spliceosome. Recently, PUF60 haploinsufficiency has been discussed as the best candidate gene for the 8q24.3 microdeletion syndrome phenotype, in seven patients with developmental delay (DD), post-natal proportionate growth retardation, and facial dysmorphism. Variable features comprised ocular coloboma, joint laxity and/or dislocation, vertebral anomalies, branchial anomalies, cardiac, and renal defects. To date, a unique patient has been reported with a *de novo* probably pathogenic mutation in PUF60 (p.His169Tyr) associated with DD, microcephaly, craniofacial and cardiac defects. We report on three additional patients carrying a *de novo* heterozygous mutation in PUF60 identified by whole exome sequencing (WES), including a splice-site mutation (c.24+1G>C), a nonsense (p.Arg448\*), and a missense change (p.Val483Ala). All four mutated patients share a core facial gestalt that was present in patients with 8q24.3 microdeletions, associated with DD. Other findings included feeding difficulties (3/4), cardiac defects (3/4), IUGR (3/4), hip dislocation (2/4), vertebral anomalies (1/4), distal anomalies (3/4), bilateral microphthalmia and irido-retinal colobomas (1/4), and branchial arch defect (1/4). These results confirm the major role of PUF60 in the 8q24.3 microdeletion phenotype since patient with a microdeletion share common manifestations with patients carrying a point mutation. PUF60 can be added to the list of genes causing a recognizable form of syndromic intellectual disability, as well as to the list of syndromic colobomas.

## P08.42

### Diagnosis of intellectual disability by sequencing all genes known in pathology.

*B. Keren<sup>1</sup>, C. Mignot<sup>2</sup>, C. Nava<sup>1</sup>, E. Lejeune<sup>3</sup>, C. Mach<sup>3</sup>, C. Estrade<sup>3</sup>, V. Olin<sup>3</sup>, A. Lafitte<sup>3</sup>, S. Karagic<sup>3</sup>, A. Jacquette<sup>2</sup>, P. Charles<sup>2</sup>, S. Whalen<sup>2</sup>, I. Marey<sup>2</sup>, S. Heide<sup>3</sup>, F. Moche<sup>3</sup>, A. Afenjar<sup>4</sup>, D. Doummar<sup>5</sup>, T. Billette de Villemeur<sup>5</sup>, A. Guet<sup>3</sup>, D. Rodriguez<sup>5</sup>, M. Marie-Laure<sup>5</sup>, Y. Marie<sup>6</sup>, S. Chantot-Bastaraud<sup>7</sup>, R. Touraine<sup>8</sup>, F. Lesne<sup>2</sup>, A. Faudet<sup>2</sup>, F. Soubrier<sup>3</sup>, D. Héron<sup>2</sup>, C. Depienne<sup>1</sup>,*

<sup>1</sup>Département de Génétique, ICM UPMC, Inserm UMR\_S1127/CNRS UMR 7225, GH Pitié-Salpêtrière, APHP, Paris, France, <sup>2</sup>Département de Génétique, centre de référence déficience intellectuelle de causes rares, filière Défiscience, GH Pitié-Salpêtrière, APHP, Paris, France, <sup>3</sup>Département de Génétique, GH Pitié-Salpêtrière, APHP, Paris, France, <sup>4</sup>Département de Génétique, centre de référence déficience intellectuelle de causes rares, filière Défiscience, GH Armand-Trousseau, APHP, Paris, France, <sup>5</sup>Service de Neuropédiatrie, GH Armand-Trousseau, APHP, Paris, France, <sup>6</sup>ICM, plateforme de génotypage et séquençage, GH Pitié-Salpêtrière, Paris, France, Paris, France, <sup>7</sup>Laboratoire de Cytogénétique, GH Armand-Trousseau, APHP, Paris, France, <sup>8</sup>Service de Génétique, CHU Nord, Saint-Etienne, France.

The etiologies of intellectual disability (ID) are very heterogeneous. For this reason, exome sequencing is increasingly used in the diagnosis of ID.

We sequenced 151 index cases with the Illumina TruSight One panel which targets the coding sequences of 4813 genes involved in human pathology. Our aim was a diagnostic yield close to exome sequencing but less expensive. All patients had a negative diagnostic work-up before sequencing including chromosome microarray analysis.

A definitive or likely diagnosis was found for 42 index cases (28%) in 39 different genes: 30 were autosomal dominant (*de novo* or with a low mosaicism in one parent), 6 were autosomal recessive, 6 were X-linked. We also made 4 partial diagnoses which do not explain the whole phenotype. Besides, 10 variants were classified as variants of unknown significance. Finally, we identified 7 incidental findings irrelevant with the indication.

In our series, 56 patients had autism and ID with normal motor development and autistic regression. In these patients, we achieved a unique diagnosis (1.7%); therefore our diagnostic yield was 42% among our 95 patients with ID and no autism. We had also 35% diagnoses in our 40 patients with ID and epilepsy.

Overall, this technique provides a significant advance in the diagnostic strategy of patients with ID. However, it highlights the need to better characterize its clinical indications. Mostly, our experience suggests that ID as part of an autistic syndrome is currently not a good indication due to the supposed multifactorial nature of this pathology.

## P08.43

**A novel mutation (c.132 G>C) in PQBP1 gene is linked to severe to mild intellectual disability in five male cousins**M. M. Alwasiyah<sup>1,2</sup>, B. Al-wasiyah<sup>3</sup>, C. Trujillo<sup>4</sup>, H. Bouazzi<sup>5</sup>;<sup>1</sup>Aziziah Maternity & Children Hospital, Jeddah, Saudi Arabia, <sup>2</sup>Center of Excellence in Genomic Medicine Research (CEGMR), King Abdullah University, Jeddah, Saudi Arabia,<sup>3</sup>King Abdullah University, Jeddah, Saudi Arabia, <sup>4</sup>Erfan & Bagedo Hospital, Jeddah, Saudi Arabia, <sup>5</sup>Necker-Enfants malades Hospital, Paris descartes University, Paris, France.

**Introduction:** Ten percent of intellectual disabilities are related to genes on the X chromosome. Among the 1200 genes of the X chromosome, 115 of them so far are X-linked intellectual disability genes (X-LID). PQBP1 gene is well known to be involved in syndromic and non-syndromic X-LID. Here we report a French family with 5 affected males who showed severe to mild intellectual disability associated with developmental delay. Subsequent genetic analysis with X exome sequencing has revealed a novel missense mutation, c.132G>C. Which was predicted to be deleterious by Polyphen-2 software. This mutation was inherited by all the affected relatives. The mothers and the grandmother have been found heterozygous for the mutation.

**Material and methods:** NGS for XLID was performed for 2 patients using SOLiD 5500 sequencer (Life technologies, Grand Island, NY, USA). Sorting and calling of SNP/InDel were performed using SAMTOOL and GATK software. All sequence variants were prioritized by scoring phylogenetic conservation and functional impact (SIFT and Polyphen-2). Candidate variants were confirmed by Sanger sequencing.

**Results:** Sequencing of X-exome from all patients identified three missense variants (c.731G>A/G244Q, c.86-88del/K29del and -c.132G>C- R10P) respectively in three different genes (TLR8, SPANXN4, and PQBP1).

**Conclusion:** We have identified three candidate genes for this family (PQBP1, TLR8 and SPANXN4), whose mutations are respectively (p.R10P, p.G244Q and p.K29del). To our knowledge these mutations are new, they segregate with the disease phenotype, and are absent in healthy subjects. The only gene known for its involvement in the ID is PQBP1

## P08.44

**Homozygote RTTN mutation in a patient with microcephaly and intellectual disability**E. Yasar<sup>1</sup>, G. Yigit<sup>2</sup>, Y. Li<sup>3</sup>, J. Altmuller<sup>3</sup>, P. Nurnberg<sup>3</sup>, S. Karatoprak<sup>4</sup>, U. Kornak<sup>5,6,7</sup>, B. Wollnik<sup>2</sup>, I. Tekedereli<sup>1</sup>;<sup>1</sup>Medical Genetics Division, Inonu University School of Medicine, Malatya, Turkey,<sup>2</sup>Institute of Human Genetics, University of Göttingen, Göttingen, Germany, <sup>3</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>4</sup>Child and Adolescent Psychiatry Division, Inonu University School of Medicine, Malatya, Turkey, <sup>5</sup>Institute of Medical Genetics and Human Genetics, Charité - Universitätsmedizin Berlin, Berlin, Germany, <sup>6</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>7</sup>Berlin-Brandenburg Center for Regenerative Therapies, Charité - Universitätsmedizin Berlin, Berlin, Germany.

**Introduction:** Primary microcephaly is an important clinical symptom associated with various genetic disorders and can be found isolated or as one of the features of a syndrome. Microcephaly and intellectual disability usually present together and more than 300 OMIM entries are described with the coexistence of these two features. In this report we describe a patient with microcephaly, deficiency of speech development, delayed motor skills, seizures and a homozygous RTTN gene mutation.

**Materials and Methods:** Cranial MRI, EEG, echocardiography, abdominal USG, visual evoked potential (VEP) and brainstem auditory evoked response (BAER) tests were performed. Karyotyping, FISH analysis and whole exome sequencing (WES) were performed.

**Results:** The index patient was 9 years and presented with primary microcephaly, developmental delay, recurrent seizures, and intellectual disability. Cranial MRI showed pachygryria, neuronal migration anomaly and cerebro-cerebellar atrophy. EEG showed frontotemporal epilepsy. Echocardiography, abdominal ultrasonography, VEP and BAER tests were normal. The patient had normal karyotype and FISH analysis for Prader Willi/Angelman Syndrome. WES analysis revealed a causative homozygous missense (c.2796A>T) RTTN gene mutation.

**Conclusions:** RTTN gene encodes rotatin protein which is localized at the ciliary basal bodies. In mice, rotation protein defects result in abnormal axial rotation and neural tube differentiation. Even though specific functions in human are still unknown, it is suggested that rotatin has similar effects on the development of the neural tube and the notochord. Since clinical variability in patients carrying autosomal recessive RTTN mutations has been described, it is important to further define the clinical spectrum of RTTN-associated disorders.

## P08.45

**Epilepsy is not a mandatory feature of STXBP1 associated Ataxia-Tremor-Retardation Syndrome**S. Beck-Woedt<sup>1</sup>, A. Riess<sup>1</sup>, U. Grasshoff<sup>1</sup>, M. Sturm<sup>1</sup>, F. Akmut<sup>1</sup>, M. Schöning<sup>2</sup>, A. Tzschach<sup>3</sup>, O. Riess<sup>1</sup>, J. Gburek-Augustat<sup>2</sup>;<sup>1</sup>Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen, Germany, <sup>2</sup>Children's Hospital; Paediatric Neurology and Developmental Medicine, University of Tübingen, Tuebingen, Germany, <sup>3</sup>Institute of Clinical Genetics, Technische Universität Dresden, Dresden, Germany.

**Introduction:** Mutations in the *STXBP1* gene were first described to cause Ohtahara syndrome (Early infantile epileptic encephalopathy, EIEE) characterized by very early infantile epileptic encephalopathy with frequent tonic spasms and a suppression-burst pattern on electroencephalogram. In the following years a wider phenotype was recognized having milder forms of epilepsies.

**Material and Methods:** More than 150 patients with intellectual disability (ID) and movement disorders, in whom genomic imbalances and fragile X syndrome had been excluded, were screened for exonic variants using the TruSight One enrichment panel (Illumina).

**Results:** Here, we present three female patients with an ataxia-tremor-retardation syndrome caused by a *de novo* dominant *STXBP1* mutation. Two of the girls were diagnosed through next-generation-sequencing as mutations in *STXBP1* were not suspected. The third patient was diagnosed by targeted genetic testing due to its clinical features strikingly similar to the first two girls. All patients showed intellectual disability and movement disorders. Additional behavioral disturbances such as hyperactivity, stereotypic behavior, hand biting and hyperventilation burst were also recognized in affected patients with *STXBP1* mutations

**Conclusion:** In summary, the characteristic feature of our three patients is the lack of epilepsy which is in contrast to the majority of the patients. Hence, epilepsy is not a mandatory feature of patients with a *STXBP1* mutation.

## P08.46

**A microdeletion at Xp11.22 detected by whole exome sequencing confirms SHROOM4 association with Stocco dos Santos syndrome and XLID in a large Greek kindred**B. Hagnefelt<sup>1</sup>, C. Konialis<sup>1</sup>, K. Lilakos<sup>2</sup>, S. Karapanou<sup>1</sup>, C. Pangalos<sup>1</sup>;<sup>1</sup>InterGenetics-Diagnostic Genetics Center, Athens, Greece, <sup>2</sup>Department of Haematology, University of Athens Medical School, Athens, Greece.

**Introduction:** The direct implication of several X-linked genes in XLID has been questioned, mainly due to the lack of replication of the particular finding and to inherent difficulties in validating *de novo* variants. We present the identification of a microdeletion encompassing the SHROOM4 gene, detected through whole exome sequencing in a large Greek kindred with syndromic XLID, thus establishing the involvement of SHROOM4 in Stocco dos Santos Syndrome and syndromic XLID.

**Materials and Methods:** A 45 yr old male, presenting with mild dysmorphic features, kyphosis, short stature, moderate intellectual disability and with four other similarly affected male relatives, was referred for genetic testing. Common genetic causes had been previously excluded. Whole exome sequencing (WES) was performed on an Ion Proton PI chip, followed by variant prioritization utilizing a custom analysis pipeline, including CNV detection through a modified coverage analysis plug-in.

**Results:** Combined analysis of WES data from the proband revealed a highly probable deletion of the Xp11.22 chromosomal region, approximately 1,85Mb in size encompassing OMIM genes SHROOM4 and BMP15. Subsequent aCGH analysis confirmed the presence of the microdeletion in 3 other similarly affected males in the family and the expected maternal segregation in the kindred.

**Conclusions:** The findings presented herein provide important evidence confirming, the hitherto debatable, involvement of the SHROOM4 gene in syndromic XLID and its association with Stocco dos Santos syndrome. Furthermore, the employed data analysis pipeline illustrates the power of WES analyses for the detection of constitutional CNV's, particularly those associated with X-linked genetic disorders.

## P08.47

### A novel splicing mutation in the IQSEC2 gene that modulates the phenotype severity in a family with intellectual disability

I. Madrigal<sup>1</sup>, M. Alvarez-Mora<sup>1</sup>, J. Rossell<sup>2</sup>, L. Rodriguez-Revenga<sup>1</sup>, O. Karlberg<sup>3</sup>, S. Sauer<sup>4</sup>, A. Syvänen<sup>3</sup>, M. Mila<sup>1</sup>;

<sup>1</sup>Hospital Clinic, Barcelona, Spain, <sup>2</sup>Hospital Universitari Son Espases, Palma de Mallorca, Spain, <sup>3</sup>Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, Sweden, <sup>4</sup>Max-Planck Institute for Molecular Genetics, Berlin, Germany.

**Introduction:** The IQSEC2 is known to have a significant role in cytoskeletal organization, dendritic spine morphology and synaptic organization.

**Material and Methods:** We studied a non-consanguineous family with five affected individuals with unexplained mild to severe ID and one with learning disabilities, studied by next generation sequencing.

**Results:** Here we report on the first splicing variant in IQSEC2 (g.88032\_88033del; NG\_021296.1) that co-segregates in a family diagnosed with an X-linked form of ID. In a percentage of the cells, the variant activates an intraexonic splice acceptor site that abolishes 26 amino acids from the highly conserved PH domain of IQSEC2 and creates a premature stop codon 36 amino acids later in exon 13. Interestingly, the percentage of aberrant splicing seems to correlate with the severity of the disease in each patient. The impact of this variant in the target tissue is unknown, but we can hypothesize that these differences may be related to the amount of abnormal IQSEC2 transcript.

**Conclusions:** To our knowledge, we are reporting a novel mechanism of IQSEC2 involvement in ID. Variants that affect splicing are related to many genetic diseases and the understanding of their role in disease expands potential opportunities for gene therapy. Modulation of aberrant splicing transcripts can become a potent therapeutic approach for many of these diseases.

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## P08.48

### Mutation spectrum of KMT2D gene in Kabuki syndrome

V. Gatinois<sup>1,2,3</sup>, G. Sarrabay<sup>1,2,3</sup>, G. Boursier<sup>1,4</sup>, A. Fabre<sup>1</sup>, E. Sanchez<sup>2</sup>, D. Genevieve<sup>2,3,5</sup>, I.

Touitou<sup>1,2,3</sup>, M. Barat-Houari<sup>1,2</sup>;

<sup>1</sup>Laboratoire de génétique des maladies rares et autoinflammatoires - Hôpital A. de Villeneuve - CHU de Montpellier, Montpellier, France, <sup>2</sup>Unité INSERM U1183 - Hôpital St Eloi - CHU de Montpellier, Montpellier, France, <sup>3</sup>Université de Montpellier, Montpellier, France, <sup>4</sup>UMR CNRS 9214 / Inserm U1046 - Université de Montpellier, Montpellier, France, <sup>5</sup>Service de Génétique Médicale - Hôpital A. de Villeneuve - CHU de Montpellier, Montpellier, France.

**Introduction:** Kabuki syndrome is a rare genetic disease involving multiple malformations, intellectual deficiency and a distinctive facial phenotype. Genetic transmission of Kabuki syndrome is mainly autosomal dominant with an estimated incidence of 1 in 32,000 births. In most cases, a KMT2D point mutation is responsible of the syndrome.

**Materials and Methods:** A large cohort of French Kabuki patients was collected. For each of them, a previous clinical assessment was performed and 260 probands with a high confidence were selected. KMT2D analysis was done by Sanger sequencing. Highlighted variants were scored into a 5-categories classification of pathogenicity based on scientific literature, public mutation databases, population frequencies, mutation nature, family segregation and prediction tools.

**Results:** We identified more than 200 unique pathogenic or likely pathogenic mutations among which 50 were never published before. Truncating mutations were the most common type of variations. Missense mutations showed a non homogenous distribution with high density in exons 50 to 53 encoding the highly conserved SET domain of KMT2D protein. Most of mutations were de novo and a very few cases showed a dominant inheritance from a parent with all or any clinical evidences of the proband. Some of these de novo mutations were recurrent highlighting the existence of mutational hot spots on KMT2D.

**Conclusions:** This large cohort allowed us to collect and classify a large number of unpublished mutations. This work has been shared on a public LOVD-database (<http://databases.lovd.nl/shared/genes/KMT2D>) in order to improve both interpretation of KMT2D variants and management of Kabuki patients.

## P08.49

### KAT6A truncating mutation in a girl with global developmental delay and a distinctive phenotype

M. Palomares Bralo<sup>1,2,3</sup>, S. García Miñáur<sup>1,2,3</sup>, L. Fernández<sup>1,2,4</sup>, E. Vallespín<sup>5,6,4</sup>, Á. del Pozo Maté<sup>1,6,4</sup>, K. Ibáñez Garikano<sup>5,6,4</sup>, J. Silla<sup>1,6,4</sup>, F. Santos-Simarro<sup>1,6,4</sup>, R. Mena<sup>5,6,4</sup>, R. Martín Arenas<sup>1</sup>, P. Tirado Requero<sup>7</sup>, E. Mansilla<sup>1,6,4</sup>, J. Nevado<sup>1,6,4</sup>, L. Alba Valdivia<sup>5</sup>, M. de Torres<sup>1,6,4</sup>, P. Lapunzina Badía<sup>1,6,4</sup>;

<sup>1</sup>INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red Enfermedades Raras, Madrid, Spain, <sup>4</sup>Centro de Investigación en Red Enfermedades Raras, Madrid, Spain, <sup>5</sup>INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>6</sup>Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain, <sup>7</sup>Servicio de neurología infantil, Hospital Universitario La Paz, Madrid, Spain.

**Background and aims:** Implementation of exome sequencing in individuals with intellectual disability syndromes has enabled the identification of de novo heterozygous mutations in up to 40% of patients with severe ID. Recently, heterozygous truncating mutations in KAT6A have been reported in 10 individuals by two independent groups as the cause of a distinct ID syndrome. Common features included global developmental delay, hypotonia, speech delay, microcephaly and/or craniosynostosis and craniofacial dysmorphisms. Here we present a girl with a de novo heterozygous mutation in KAT6A with a phenotype that resembles the one reported in previous cases, reinforcing the association between KAT6A mutations and a new recognizable syndrome.

**Method:** Whole exome sequencing was performed as a trio in this family in an attempt to identify de novo mutations causing the ID and the peculiar phenotype observed in this girl. At 22 months she presented with growth and developmental delay, congenital heart defects, microcephaly and distinctive facial features. Initial investigations (cerebral MRI, skeletal survey, abdominal US scan, metabolic screen, standard chromosome analysis and a 60K customized aCGH) were all normal.

**Results:** A de novo mutation in KAT6A was detected: NM\_001099412.1:c.4228\_4232delAAAGA(p.Lys1410Glyfs\*7). This change was confirmed by Sanger sequencing.

**Discussion and conclusion:** This case contributes to delineate the phenotype of a new intellectual disability syndrome associated with KAT6A mutations and highlights the potential of whole exome sequencing for the identification of causal mutations in individuals with intellectual disability where no diagnosis has been reached after routine investigations.

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## P08.50

### KCNK9 imprinting syndrome - a treatable disorder?

J. M. Graham<sup>1</sup>, N. Zadeh<sup>2</sup>, E. Tan<sup>3</sup>, M. Deardorff<sup>4</sup>, L. Sagi-Dain<sup>5</sup>, S. Shalev<sup>6</sup>;

<sup>1</sup>Cedars Sinai Medical Center, Los Angeles, CA, United States, <sup>2</sup>CHOC Children's Hospital, Orange, CA, United States, <sup>3</sup>KK Women's and Children's Hospital, Singapore, Singapore, <sup>4</sup>Children's Hospital of Philadelphia, Philadelphia, PA, United States, <sup>5</sup>Emek Medical Center, Afule, Israel, <sup>6</sup>Rapaport Faculty of Medicine, Technion, Haifa, Israel.

Barel et al. (2008) mapped a new KCNK9 imprinting syndrome to chromosomal region 8q24 in a large Arab Israeli family, and demonstrated that this syndrome is caused by a specific missense mutation 770G>A in exon 2, replacing glycine at position 236 by arginine (G236R) in the maternal copy of KCNK9 within this locus. KCNK9 (also called TASK3) encodes a member of the two pore-domain potassium channel (K2P) subfamily. This gene is normally imprinted with paternal silencing, thus a mutation in the maternal copy of the gene will result in disease, whereas a mutation in the paternal copy will have no effect. Exome sequencing in 4 other patients with developmental delay and central hypotonia revealed de novo G236R mutations. These patients demonstrated congenital hypotonia, variable cleft palate, normal MRIs and EEGs, delayed development, and severe feeding problems. Associated facial dysmorphic features included dolichocephaly with bitemporal narrowing, short philtrum, tented upper lip, palatal abnormalities, and small mandible. Features in older members of the original Arab Israeli family included intellectual disability of variable severity, severe feeding difficulties in infancy with dysphagia of liquids and dysphonia with a muffled voice into early adulthood, generalized hypotonia, weakness of proximal muscles, elongated face with narrow bitemporal diameter, and reduced facial movements. We describe the clinical features in the 4 recently recognized patients and compare them with those found in members of the original Arab-Israeli family and suggest this may be a treatable disorder.

**P08.51****About a case with a novel KIRREL3 variant: further clinical delineation of the associated phenotype**

**E. Ranza<sup>1</sup>, M. Guipponi<sup>1</sup>, J. L. Blouin<sup>1</sup>, P. Makrythanasis<sup>1</sup>, C. Gehrig<sup>1</sup>, C. Menache<sup>2</sup>, A. Bottani<sup>1</sup>, A. Martin<sup>3</sup>, M. E. Williams<sup>3</sup>, S. E. Antonarakis<sup>1,4</sup>,**  
<sup>1</sup>HUG, University Hospitals of Geneva, Geneva, Switzerland, <sup>2</sup>Neuropediatrics, Clinique des Grangettes, Chêne-Bougeries, Switzerland, <sup>3</sup>Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT, United States, <sup>4</sup>Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland.

KIRREL3 is a cell-adhesion molecule of the immunoglobulin superfamily, that has been implicated in cerebral development, synaptic maintenance and plasticity. It interacts with neuronal proteins, including the synaptic scaffolding protein CASK, which is associated with an X-linked intellectual disability (ID) syndrome (OMIM#300749).

To date, only a few patients with pathogenic variants in *KIRREL3* have been reported (OMIM#612581): their phenotype was described as mild to severe ID, without further details, highlighting the need for a better clinical delineation of this syndrome.

Here, we report a case of a 12 year-old male with moderate ID, obesity, ADHD and behavioural problems, mostly hyperphagia. The patient also had mild dysmorphic features, such as enophthalmia, bulbous nose, short philtrum, thin upper lip and wide spaced teeth with two missing superior canines. Brain MRI revealed wide asymmetrical ventricular spaces.

Array-CGH, *FMRI* testing and 15q11.2 DNA methylation were all normal. Exome sequencing followed by targeted analysis of a panel of 990 ID genes, identified a novel *de novo* missense variant in *KIRREL3* c.2019G>A:p.(Met673Ile) which was classified as likely pathogenic according to the latest ACMG guidelines. The functional impact of the mutant *KIRREL3* on synapse development in neuronal cells is currently ongoing.

This study will provide insight into the clinical spectrum associated with pathogenic variants in *KIRREL3* and the molecular mechanisms underlying *KIRREL3*-related neurodevelopmental disorders.

**P08.52****Efficacy of GenIDA, a family-oriented international online registry and clinical database for genetic forms of intellectual disability and/or autism, to collect medically relevant information on the Koolen - de Vries Syndrome**

**F. P. Colin<sup>1</sup>, K. P. J. Schellekens<sup>2</sup>, T. Mazzucotelli<sup>1</sup>, T. Kleefstra<sup>2</sup>, D. A. Koolen<sup>2</sup>, J. L. Mandel<sup>1,3,4</sup>,**

<sup>1</sup>Translational medicine and neurogenetics dept., Institut de génétique et de biologie moléculaire et cellulaire (IGBMC) – INSERM U964 – CNRS UMR7104 – University of Strasbourg, Illkirch, France, <sup>2</sup>Department of Human Genetics, Radboud University Medical Centre, Nijmegen, Netherlands, <sup>3</sup>Genetic diagnostic laboratory, CHU, Strasbourg, France, <sup>4</sup>Collège de France, Chaire de génétique humaine, Paris, France.

Intellectual disability (ID) and autism spectrum disorders (ASD) are overlapping disorders that constitute a major public health problem with a cumulated frequency of about 2.5%. Progress in genome analysis has allowed the identification of many recurrent CNVs and more than 600 genes implicated in monogenic forms of ID/ASD. However, information on the clinical spectrum and natural history is often lacking behind. These data are essential for improved clinical management of patients and genetic counselling of family members.

Therefore, we initiated a unique database model for specific genetic causes of ID/ASD, called GenIDA (genida.unistra.fr), whereby clinical information is entered and updated by the family of the affected individual based on a structured clinical questionnaire that is available in multiple languages. We have used Koolen - de Vries syndrome (KdVS) as a test case for the GenIDA approach. KdVS is a multi system condition characterized by (neonatal) hypotonia, moderate ID, epilepsy, congenital abnormalities and characteristic facial dysmorphism. We transferred a large existing family-based KdVS clinical dataset (>70 cases) and asked parents to update the data on the GenIDA website.

This project shows the willingness of parents to participate in studies dealing with rare diseases affecting their child. Direct comparison with data collected by clinicians allowed us to evaluate the quality of the data entered by families, search for novel and/or more penetrant comorbidities (i.e. behavioral problems like hyperactivity were significantly more reported) and generate a first natural history analysis.

- University of Strasbourg Institute of Advanced Study

- Fondation University of Strasbourg

**P08.53****Unreported mutation found by whole exome sequencing in the KPTN gene is causative for Macrocephaly in an Iranian infant**

**H. Galehdari<sup>1</sup>, G. Shariati<sup>2</sup>, A. Saberi<sup>3</sup>, M. Hamid<sup>4</sup>, E. Jahangiri<sup>1</sup>, F. Seifi<sup>1</sup>, N. Mazaheri<sup>1</sup>, M. Zamani<sup>1</sup>, J. Zeighami<sup>1</sup>, M. Sarvari<sup>1</sup>,**  
<sup>1</sup>Genetics, Ahwaz, Iran, Islamic Republic of, <sup>2</sup>Narges Genetic Diagnostic Lab, Ahwaz, Iran, Islamic Republic of, <sup>3</sup>Jondishapur University, Ahwaz, Iran, Islamic Republic of, <sup>4</sup>Pasteur Institute, Tehran, Iran, Islamic Republic of.

Mutation in KPTN gene have been identified as causative for autosomal recessive mental retardation and clinically characterized by significantly below average general intellectual functioning associated with impairments in adaptive behavior and manifested during the developmental period. Most consistent features are global developmental delay, macrocephaly with frontal bossing, high levels of anxiety, repetitive speech, and mild to severe speech deficits. Recent reports suggest that KPTN mutations are associated with a broader phenotypic spectrum. We used next generation sequencing to find out disease causing gene confirmed by Sanger sequencing. We identified a novel nonsense mutation(c.609 G>A) in 14 years old Iranian patient with autistic behavior, macrocephaly, skull deformity, speech impairment and symptoms included hydrocephaly have been showed by MRI at birth. This mutation was neither found in ExAC nor 1000G and ClinVar.

**P08.54****Exome sequencing reveals LINGO1 causative variants in autosomal recessive intellectual disability and developmental delay**

**M. Ansar<sup>1</sup>, S. Riazuddin<sup>2</sup>, P. Makrythanasis<sup>1,3</sup>, M. T. Sarwar<sup>4</sup>, Z. Iqbal<sup>5,6</sup>, J. Khan<sup>4</sup>, S. A. Paracha<sup>4</sup>, M. Hussain<sup>5,7</sup>, A. Razzaq<sup>5,7</sup>, D. L. Polla<sup>5</sup>, A. Suhail<sup>4</sup>, A. Holmgren<sup>8</sup>, D. Misceo<sup>9</sup>, A. P. M. de Brouwer<sup>5</sup>, M. Guipponi<sup>1,3</sup>, F. A. Santoni<sup>1,2</sup>, E. Frengen<sup>8</sup>, J. Ahmed<sup>4</sup>, H. van Bokhoven<sup>5</sup>, S. Riazuddin<sup>9,10</sup>, S. E. Antonarakis<sup>1,3</sup>,**

<sup>1</sup>Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland, <sup>2</sup>Department of Otorhinolaryngology-Head & Neck Surgery, School of Medicine, University of Maryland, Baltimore, MD, 21201 USA, Baltimore, MD, United States, <sup>3</sup>Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland,

<sup>4</sup>Institute of Basic Medical Sciences (IBMS), Khyber Medical University, Peshawar, Pakistan, <sup>5</sup>Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, Netherlands, <sup>6</sup>Department of Neurology, Oslo University Hospital, Oslo, Norway, <sup>7</sup>National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, <sup>8</sup>Department of Medical Genetics, Oslo University Hospital, University of Oslo, Oslo, Norway, <sup>9</sup>Allama Iqbal Medical College, University of Health Sciences, Lahore, Pakistan, <sup>10</sup>Shaheed Zulfiqar Ali Bhutto Medical University, Pakistan Institute of Medical Sciences, Islamabad, Pakistan.

LINGO1, a transmembrane receptor, is predominantly expressed in central nervous system and is involved in the inhibition of neuronal myelination, through activation of the NgR1 signaling pathway. Various GWAS and expression studies have implicated increased expression of this gene with Multiple Sclerosis, Essential Tremor and Parkinson's disease. We have studied two unrelated Pakistani consanguineous families from different areas of the country. Eleven patients of both families presented with moderate to severe intellectual disability, speech and motor delay. Out of 11 patients, four presented with microcephaly and seven with generalized tonic-clonic seizures. We performed exome sequencing in order to identify the potential causal variant. We have identified a homozygous missense variant (NM\_032808.6:c.869G>A:p.(Arg290His)) in family one and different homozygous variant in family two (NM\_032808.6:c.863A>G p.(Tyr288Cys)) in LINGO1, that were predicted to be pathogenic by SIFT, PolyPhen and MutationTaster, and segregated with the phenotype in the respective families. NgR1 signaling pathway mediated by LINGO1-NgR1 complex, negatively regulates oligodendrocyte differentiation and neuronal survival. Previously mouse and zebrafish models have been described and presented the role of LINGO1 in abnormal neuronal development and central nervous system myelination. Taken together, our results indicate that LINGO1 missense variants cause autosomal recessive intellectual disability. However, further functional studies are warranted to dissect the exact role of the identified variants.

**P08.55****Recurrence of *MEF2C* heterozygous mutation in siblings indicating parental germline mosaicism**I. K. Nielsen<sup>1</sup>, I. S. Pedersen<sup>2</sup>, V. Q. Le<sup>2</sup>, A. Ernst<sup>2</sup>, J. R. Ostergaard<sup>3</sup>;<sup>1</sup>Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark,<sup>2</sup>Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark, <sup>3</sup>Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark.

Introduction: The autosomal dominant mental retardation syndrome-20 (MRD20) is caused by a heterozygous mutation in the *MEF2C* gene and characterized by severe intellectual disability with absent speech, limited walking abilities, hand stereotypies, epilepsy, and lack of major malformations. *MEF2C* is essential for early neurogenesis, neuronal migration and differentiation of the brain. To date, all individuals reported have been simplex cases, resulting from de novo deletions or point mutations. We report a case with putative gonadal mosaicism in a parent leading to siblings with MRD20.

Material and methods: Female siblings (2 and 10 yrs) of healthy unrelated parents. The children had a similar phenotype with severe intellectual disability, absent spoken language, few seizures, stereotypic hand movements, unstable wide-based gait, and minor facial dysmorphism.

Whole exome sequencing was carried out and mutations were validated by Sanger sequencing.

Results: A pathogenic heterozygous frame-shift mutation in *MEF2C*, NM\_001193347.1:c.582delT was detected in lymphocytes from both girls. The mutation was not found in lymphocytes from any of the parents. The parents did not wish to have any other samples taken in order to confirm a somatic or germline parental mosaicism.

Conclusions: Finding the same mutation in siblings, and not in the parents, indicates parental gonadal mosaicism. To the authors' knowledge, this has not been described before for MRD20, suggesting that the recurrence risk, for parents with a child with MRD20, might be higher than by chance alone. Considerations of (and testing for) mosaicism might be considered a helpful tool in the genetic counseling of these families.

**P08.56****MEF2C mutations in the clinic - a case series**C. Breen<sup>1,2</sup>, J. Innes<sup>1,2</sup>, J. Clayton-Smith<sup>1,2</sup>, E. A. Jones<sup>1,2</sup>, H. Kingston<sup>1,2</sup>, W. Reardon<sup>1,2</sup>, B.Kerr<sup>1,2</sup>, S. Douzgou<sup>1,2</sup>;<sup>1</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom, <sup>2</sup>Institute of Human Development Faculty of Medical & Human Sciences, University of Manchester, Manchester, United Kingdom.

Patients with chromosome 5q14.3q15 microdeletions involving the phenocritical *MEF2C* gene present with features including early and severe developmental delay including absence of speech and hypotonia; many remain unable to walk unaided. A facial gestalt includes a high and wide forehead,

pronounced eyebrows, down turned corners of the mouth, and a prominent philtrum. *MEF2C* mutations are estimated to account for 1% patients with severe mental retardation and 2% Rett-like patients, with a phenotype similar to those with the microdeletion.

We report 5 new patients seen by our team, 4 with deletions including *MEF2C* and one with a point mutation. We compare this additional phenotypic data with that reported in the literature, and including a patient with the deletion of both *MEF2C* and the adjacent *RASA1* who also presents with capillary malformation, confirming a previously reported association. We also discuss the facial phenotype of our patients, with reference to the recent literature around *MEF2C* as a transcription factor in craniofacial development.

**P08.57****Novel PGAP1 gene mutation in patients with mental retardation in a Turkish family**L. Özer<sup>1</sup>, D. Trujillano<sup>2</sup>, E. Ünsal<sup>3</sup>, S. Aktuna<sup>3</sup>, F. Akyiğit<sup>1</sup>, P. Çelikkol<sup>1</sup>, A. Rolfs<sup>4</sup>, V. Baltacı<sup>5</sup>;<sup>1</sup>Mikrogen Genetic Diagnosis Center, Ankara, Turkey, <sup>2</sup>Centogene, Rostock, Germany,<sup>3</sup>İstanbul Yeni Yüzyıl University School of Medicine, Department of Medical Biology and Genetics, İstanbul, Turkey, <sup>4</sup>Centogene, Rostock, Turkey, <sup>5</sup>İstanbul Yeni Yüzyıl University School of Medicine, Department of Medical Biology and Genetics, iSTANBUL, Turkey.

Introduction: Pathogenic variants in the *PGAP1* gene were reported as causative for autosomal recessive mental retardation type 42 and hereditary spastic paraparesis type 67. Materials and Methods: Here we represent a Turkish family with two affected children who were born as the first (14 years old male) and second child (11 years old male) of consanguineous family. Growth and motor delay, delayed language development, short stature, intellectual disability, stereotypic movements, autistic features, large ears, abnormality of the pinna, flattened nasal bridge, shortening of all distal phalanges, clinodactyly of 5th finger were noted in two patients. The second child had epileptic seizures which were not seen in first child. Cranial MRI of first child revealed bilateral myelinisation delay at temporal lobes, thinning of corpus callosum. Whole exome sequencing (WES) was performed for patients and their parents. Results: WES analysis revealed homozygous previously unreported variant in exon 25 of the *PGAP1* gene, c.2349delins14 (p.His783Glnfs\*2) in patients. Both parents are heterozygous carrier of the detected variant. Conclusions: We present a novel *PGAP1* variant in a Turkish family. Few cases were reported about *PGAP1* mutations in patients with intellectual disability. Our case presentation will be helpful to define the phenotype of the patients with *PGAP1* mutations and to confirm the clinical findings of previous studies and also to shed light on the further studies.

Table 1. Reported Cases with *PGAP1* Mutations:

PATIENT	AGE	SEX	ORIGIN	CLINICAL FEATURES	MUTATION	REFERENCES
1,2	4,2	F,M	Syrian	developmental delay, hypotonia, seizures, stereotypic movements, large ears, flattened nasal bridge	c.589_591del	Murakami et al., 2014
3,4	6, 9 months	M,M	?	spasticity, developmental delay	c.1952+1G4T	Novarino et al, 2014
5	7	M	?	cerebral visual impairment, strabismus, nystagmus, intellectual disability, hypotonia, upward slanting palpebral fissures, deep-set eyes, large ear lobes, prominent helices and antihelices, teeth showed extra mamelons with diminished enamel	c.274_276del and c.921_925del	Bosch et al, 2015
6,7	9,5	F,M	Turkish	severe psychomotor retardation, hypotonia, nystagmus, retinal dystrophy, comprised mild synophrys, low frontal and posterior hair line, hypertelorism, bifid uvula, mild pectus excavatum and broad 1st toes.	(c.1090-2A>G; IVS9-2A>G; p.?)	Granzow et al, 2015
8,9	11,14	M,M	Turkish	developmental delay, seizures, stereotypic movements, autistic features, large ears, clinodactyly, flattened nasal bridge, strabismus	c.2349delins14	<b>PRESENT CASE</b>

## P08.58

**Microdeletion located in Xq22 in a girl. Confirmation of a specific phenotype**

*F. Giuliano<sup>1</sup>, H. Karmous-Benally<sup>1</sup>, M. Dayem-Quere<sup>1</sup>, C. Desaldeleer<sup>2</sup>, C. Costet-Fighiera<sup>3</sup>, G. Pitelet<sup>2</sup>;*

<sup>1</sup>Department of Medical Genetics, Centre Hospitalier Universitaire de Nice, Nice, France,

<sup>2</sup>Department of Pediatrics, Hôpitaux Pédiatriques de Nice, CHU Lenal, Nice, France,

<sup>3</sup>Consultation of Ophthalmology, Clinique Saint George, Nice, France.

Alteration of proteolipid protein 1 gene (PLP1) mapped in Xq22 is usually known to lead to an X-linked hypomyelination disorder, Pelizaeus Merzbacher disease (PMD; MIM =312080). Mutations and duplications are the main causes of PLP dysfunction in affected patients. Rare deletions have been described, all of them of small size.

Five females with microdeletion, exactly mapped in Xq22 are reported [Yamamoto et al, 2014], but do not share the similar features as PMD male patients. However, they appear to share a similar phenotype, including severe developmental delay, major sleep disturbance, similar facial features, ophthalmologic abnormalities and feeding difficulties. Cerebral MRI may show thin corpus callosum and for some delayed myelination.

We identified a female patient with de novo copy number aberration located in Xq22. We compare our clinical, biological and radiological data with the five affected ones previously described by Yamamoto et al, aiming to demonstrate that Xq22 deletions in female should be considered as responsible for a specific phenotype.

## P08.60

**Diagnostic challenges due to mosaics**

*C. Rusu<sup>1</sup>, A. Sireteanu<sup>2</sup>, M. Gramescu<sup>1</sup>, R. Popescu<sup>1</sup>, E. Gorduza<sup>1</sup>;*

<sup>1</sup>University of Medicine and Pharmacy, Iasi, Romania, <sup>2</sup>Regional Institute of Oncology, Iasi, Romania.

We present 4 different cases to illustrate diagnostic challenges and genetic counseling issues raised by mosaics.

Case 1: 10 years old male, first child of a young, healthy, unrelated couple. No family history. Physical examination reveals: hypertelorism, hypospadias and developmental delay. Brain MRI identified Dandy-Walker malformation. Clinical suspicion of Opitz G/BBB syndrome was raised, but molecular test was normal. In time, areas of dry skin along Blaschko lines became evident and new DNA test identified an X chromosome microdeletion in mosaic.

Case 2: 13 years old female, the only child of a young, healthy, unrelated couple. As an infant she presented with milia, cleft palate, lobulated tongue and digital anomalies, features highly suggestive for OFD1 syndrome, but molecular testing was normal. With age, pigmentary areas along Blaschko lines became evident, suggesting the presence of a mosaic.

Case 3: 3 years old macrosomic female that associates mild dysmorphic face, blindness, deafness, seizures and severe intellectual disability. The presence of the mosaic was suggested by 2 small skin defects. Blood investigations have been normal, but DNA investigations from oral cells revealed 12p tetrasomy and established the diagnosis of Pallister-Killian syndrome.

Case 4: 5 years old male with mild dysmorphic face, very deep palmar and plantar creases, cryptorchidism and developmental delay. The karyotype revealed trisomy 8 in mosaic (5 lines).

In conclusion, we present 4 cases with mosaics to illustrate suggestive features and to discuss challenges related to laboratory diagnosis and genetic counseling.

## P08.61

**Genetic analysis of delayed motor mental development and Unverricht-Lundborg disease in a large highly consanguineous family from Turkey**

*E. Yucesan<sup>1</sup>, F. Tuncer<sup>1</sup>, M. Calik<sup>2</sup>, N. Bebek<sup>3</sup>, U. Ozbek<sup>1</sup>, S. Ugur Iseri<sup>1</sup>;*

<sup>1</sup>Institute for Experimental Medicine, Istanbul, Turkey, <sup>2</sup>Harran University, Harran Faculty of Medicine, Sanliurfa, Turkey, <sup>3</sup>Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey.

Combined analysis of homozygosity mapping and whole exome sequencing (WES) are powerful tool to detect pathogenic variants associated with recessive disorders. Herein, WES and SNP array used for clarifying delayed motor mental development (DMMD). We also conducted genetic test for Unverricht-Lundborg Disease (ULD) in family for different individuals.

## Introduction

We ascertained consanguineous family presenting two different phenotype, DMMD, Unverricht-Lundborg Disease (ULD) respectively. In this study, we applied two-step approach, where SNP array analysis, defining homozygosity regions were used to limit exome variants to specific chromosomal regions. Also mutation analysis of CSTB for ULD.

## Materials and Methods

We analyzed consanguineous family with two affected children having DMMD. Other two affected children diagnosed with ULD. 2 affected sibs were genotyped using SNP array. Homozygous regions were detected using PLINK. WES was performed for 2 affected sibs. In addition, analysis of CSTB gene was performed for promoter region via longPCR, as mutations in CSTB have been implicated in ULD.

## Results

According to previous results, homozygous haplotypes were valid for 6 distinct chromosomes. Variants filtered from WES data solely for homozygous regions. Amongst, novel variants with pathogenic affect on protein were prioritized. Evaluating homozygous haplotypes for filtered variants, segregation analysis in the family is continuing.

LongPCR analysis revealed repeat expansion of almost 60 copies in promoter region in ULD

## Conclusions

Parallel methods used to explain genetic background of DMMD and preliminary analysis are still continuing. Also we detected repeat expansion in CSTB gene's promoter for affected sibs.

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## P08.62

**Expanding the phenotype associated with Naa10 related N-terminal acetylation deficiency**

*C. Saunier<sup>1</sup>, S. Støve<sup>2</sup>, B. Popp<sup>3</sup>, B. Gérard<sup>4</sup>, M. Blenski<sup>5</sup>, N. AhMew<sup>6</sup>, C. de Bie<sup>7</sup>, P. Goldenberg<sup>8</sup>, B. Isidor<sup>9</sup>, B. Keren<sup>10</sup>, B. Leheup<sup>11</sup>, L. Lampert<sup>11</sup>, C. Mignot<sup>12</sup>, K. Tezcan<sup>13</sup>, G. Mancini<sup>14</sup>, C. Nava<sup>10</sup>, M. Wasserstein<sup>15</sup>, A. Bruel<sup>16</sup>, J. Thevenon<sup>17</sup>, A. Masurel<sup>17</sup>, Y. Duffourd<sup>16</sup>, P. Kuentz<sup>16</sup>, F. Huet<sup>16</sup>, J. Rivière<sup>16</sup>, M. Slegtenhorst<sup>14</sup>, L. Faivre<sup>16</sup>, A. Piton<sup>4</sup>, A. Reis<sup>3</sup>, T. Arnesen<sup>17</sup>, C. Thauvin-Robinet<sup>16</sup>, C. Zweier<sup>3</sup>;*

<sup>1</sup>CHU, DIJON, France, <sup>2</sup>University of Bergen, Bergen, Norway, <sup>3</sup>Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany,

<sup>4</sup>CHU, Strasbourg, France, <sup>5</sup>University of Bergen, Bergen, Norway, <sup>6</sup>Children's National Medical Center, Washington, WA, United States, <sup>7</sup>UMC Utrecht, Utrecht, Netherlands,

<sup>8</sup>Massachusetts General Hospital, Boston, MA, United States, <sup>9</sup>CHU, Nantes, France, <sup>10</sup>AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France, <sup>11</sup>CHU, Nancy, France, <sup>12</sup>APHP, Groupe Hospitalier Pitié Salpêtrière, Paris, France, <sup>13</sup>Kaiser Permanente, Sacramento, CA, United States, <sup>14</sup>Erasmus University Medical Center, Rotterdam, Netherlands, <sup>15</sup>Icahn School of Medicine at Mount Sinai, New York, NY, United States, <sup>16</sup>CHU / Université de Bourgogne, DIJON, France, <sup>17</sup>Haukeland University Hospital, Bergen, Norway.

Background: N-terminal acetylation is a common protein modification in eukaryotes associated with numerous cellular processes. Inherited mutations in NAA10, encoding the catalytic subunit of the major N-terminal acetylation complex NatA have been associated with diverse, syndromic X-linked recessive disorders, while de novo missense mutations have been reported in one male and one female individual with severe intellectual disability but otherwise unspecific phenotypes. Thus, the full genetic and clinical spectrum of Naa10 deficiency is yet to be delineated.

Methods and Results: Whole exome or panel sequencing identified three different novel and one known missense mutation in NAA10, de novo in eleven females, and due to maternal germ line mosaicism in another girl and her more severely affected and deceased brother. Common phenotypes in the affected females included severe intellectual disability and postnatal growth failure with pronounced microcephaly. In vitro enzymatic assays for the novel, recurrent mutations p.(Arg83Cys) and p.(Phe128Leu) revealed a reduced catalytic activity. X-inactivation was random in four of six tested females.

Conclusions: We report on 12 females with mutations in NAA10 and thus further expand the mutational and clinical spectrum. The core phenotype of X-linked Naa10 related N-terminal-acetyltransferase deficiency in both males and females includes developmental delay, severe intellectual disability, postnatal growth failure with severe microcephaly and skeletal or cardiac anomalies. Genotype-phenotype correlations within and between both genders are complex and may include various factors such as location and nature of mutations, enzymatic stability and activity, and X-inactivation in females.

## P08.63

**Genetic disease amongst children referred to an East London neurodevelopmental clinic over fifteen years**

*S. K. Best<sup>1</sup>, M. Bajaj<sup>1</sup>, E. Rosser<sup>2</sup>;*

<sup>1</sup>Wellington Way Children's Centre, Barts Health NHS Trust, London, United Kingdom,

<sup>2</sup>Clinical Genetics Department, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom.

Introduction: Tower Hamlets in East London is an ethnically diverse area with high levels of consanguinity. Patients referred to the neurodevelopmental clinic with developmental delay or congenital abnormalities bet-

ween 1999 and 2013 were evaluated for underlying diagnoses and the use of genetic testing.

Methods: Clinical notes were reviewed. Genetic test results were obtained. Excel analysis and Chi-squared testing was performed. Undiagnosed patients were labelled "likely" genetic disease if they had 2/3 of: developmental delay, congenital abnormality or parental consanguinity, and "highly likely" if they had 3/3. Undiagnosed patients with obvious alternative causes were not labelled.

Results: 749 patients were included. 53.8% (403) had undergone genetic testing, of which 26.2% (164) had a confirmed genetic diagnosis. 40.7% of those undergoing genetic testing had a confirmed genetic diagnosis (128/403). A further 123 patients were thought likely/very likely to have an undiagnosed genetic disorder, of which 77 (62.6%) had known consanguineous parents. Confirmed genetic diagnosis was statistically significantly more common amongst the consanguineous population than the known non-consanguineous population (43/128 vs 75/333,  $p<0.025$ ).

The proportion undergoing genetic testing increased over time. Microarray testing was the most common test used and became more prevalent after 2010.

84 different genetic diseases were confirmed. Syndromic disorders and aneuploidies were most common.

Conclusions: Genetic disease was common amongst children referred to the neurodevelopmental clinic and genetic testing is important in their evaluation. Consanguinity significantly increases the likelihood of causal genetic disease. Neurosusceptibility microdeletions/micropuplications are under-represented as microarray testing was not routinely available throughout this period.

## P08.64

### Mosaic Xq26.2q26.3 duplications associated with intellectual disability and congenital malformations: a new chromosomal syndrome?

S. G. Vorsanova<sup>1,2,3</sup>, Y. B. Yurov<sup>2,1,3</sup>, M. A. Zelenova<sup>2,1,3</sup>, O. S. Kurinnaia<sup>1,2,3</sup>, K. Vasin<sup>2,1,3</sup>, V. S. Kravets<sup>1,3,2</sup>, M. Gordeeva<sup>1</sup>, I. Y. Yurov<sup>2,1,4</sup>;

<sup>1</sup>Research and Clinical Institute for Pediatrics named after YE Veltishev at the Pirogov Russian National Research Medical University, Ministry of Health, Moscow, Russian Federation, <sup>2</sup>Mental Health Research Center, Moscow, Russian Federation, <sup>3</sup>Moscow State University of Psychology and Education, Moscow, Russian Federation, <sup>4</sup>Department of Medical Genetics, Russian Medical Academy of Postgraduate Education, Ministry of Health, Moscow, Russian Federation.

Introduction. Recurrent mosaic duplications are exclusive in the available literature. This is especially the case in terms of clinically relevant X chromosome duplications in females. However, a growing amount of reports on somatic genome variations causing intellectual disability and congenital malformations allows speculations that mosaic (sub)chromosomal rearrangements might be more common and clinically relevant than previously recognized.

Materials and Methods. Molecular karyotyping using SNP/oligonucleotide microarray (resolution: >1 kbp) was performed in a cohort of 402 children with intellectual disability, autism, epilepsy and/or congenital malformations.

Results. Four girls (~1%) have exhibited mosaic duplications spanning Xq26.2q26.3.

The age varied from 1 year 1 month to 6 years. Shared features included intellectual disability/developmental delay, microcephaly, broad phyltrum and wide nasal bridge, long lashes, ptosis; one patient demonstrated autistic features. The overlapping region for all duplications included 8 genes: *GPC3*, *MIR19B2*, *MIR106A*, *PHF6*, *HPRT1*, *MIR503*, *MIR424*, *PLAC1*. Although non-mosaic duplication in this region involving genes *PHF6* and *HPRT1* has been described in the literature previously, the lack of phenotypic resemblance to the detected cases and an original bioinformatics analysis allow the speculation that mosaic duplications Xq26.2q26.3 might be a new chromosomal syndrome associated with intellectual disability and congenital malformations.

Conclusions. Our study demonstrates that mosaic duplications Xq26.2q26.3 are relatively common in children with intellectual disability, autism, epilepsy and/or congenital malformations. Furthermore, our findings demonstrate the possibility that mosaic chromosomal rearrangements can be a cause for a new chromosomal syndrome. Supported by the Russian Science Foundation (grant: 14-15-00411).

## P08.65

### Mendeliome sequencing increases the diagnostic yield in patients with unexplained intellectual disability by 30% (a single center experience)

A. Rump<sup>1</sup>, L. Mackenroth<sup>1</sup>, A. Kahlert<sup>1</sup>, J. Lemke<sup>2</sup>, K. Hackmann<sup>1</sup>, E. Betcheva-Krajcir<sup>1</sup>, A. Krüger<sup>1</sup>, R. Opitz<sup>3</sup>, F. Kuhlee<sup>1</sup>, A. Tzschach<sup>1</sup>, E. Schröck<sup>1</sup>, N. Di Donato<sup>1</sup>;

<sup>1</sup>Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, <sup>2</sup>Institut für Humangenetik, Universitätsklinikum Leipzig, Leipzig, Germany, <sup>3</sup>Abteilung für Gynäkologie und Geburtshilfe, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.

Introduction: Despite extensive use of chromosomal microarrays, up to 50% of children with developmental delay still remain undiagnosed. Therefore, we applied mendeliome sequencing to 57 index patients with developmental delay or intellectual disability (ID) and pre-excluded genomic imbalances.

Method: Genomic DNA samples of 28 parent-child trios plus 29 individuals were analyzed for mutations in 4813 genes, using the TruSightOne gene panel on the MiSeq platform (Illumina, San Diego, CA). Sequence variants were called by two independent platforms: the GATK pipeline installed on the MiSeq and the CLC Biomedical Genomics platform (Qiagen, Hilden, Germany). All variants with putative effect on amino acid level were screened for clinical and molecular concordance (i.e. disease-association of the gene, published mutation) and all modes of inheritance (dominant de novo, autosomal recessive, X-recessive) were considered. The results were discussed in a team of clinicians and molecular geneticists and relevant variants were validated by Sanger-sequencing.

Results: Using the mendeliome in a diagnostic setting, we established a diagnosis in 16 of the 57 index patients (28%). For seven further patients, we found one or two possibly causative candidates (13%). 5 patients (9%) showed incidental findings which either made treatment or surveillance necessary (homozygous *MUTYH*-mutations, *SDHA*-mutation) or led to an increased risk for a recessive disease in children (PAH- or *CFTR*-mutations).

Conclusion: Mendeliome sequencing significantly increases the diagnostic yield in patients with ID unsolved by previous routine testing (array-CGH, karyotyping). However, variant interpretation remains challenging and requires standardized procedures for using the new technology in a standard diagnostic setting.

## P08.66

### Evaluation of non-coding copy number variants in neurodevelopmental disorders

R. Asadollahi<sup>1</sup>, A. Mihai<sup>1</sup>, B. Oneda<sup>1</sup>, R. Baldinger<sup>1</sup>, R. Reissmann<sup>1</sup>, P. Boonsawat<sup>1</sup>, M. Papik<sup>1</sup>, M. Zwieier<sup>1</sup>, S. Azzarello-Burri<sup>1</sup>, A. Baumer<sup>1</sup>, P. Joset<sup>1</sup>, K. Steindl<sup>1</sup>, A. Rauch<sup>1</sup>, Institute of Medical Genetics, University of Zurich, Zurich, Switzerland.

The genome-wide significance of non-coding CNVs has not been studied in neurodevelopmental disorders (NDDs). Therefore, by high-resolution chromosomal microarray analysis, we investigated all non-coding CNVs in 121 patients with undiagnosed NDDs. We detected 548 rare non-coding variants with a median size of 8 kb, of which 94 (~17%) were seen < 3 times in our total in-house arrays and had medium to high regulatory scores. The scoring system was based on the evolutionary conservation and affected non-coding RNAs, cis-regulatory elements and histone modification markers. Considering the pattern of inheritance and/or literature assessment, we classified 9 of 94 (~9.6%) as strong candidates of which 6 were true positive. From these variants, two (25 kb de novo and 122 kb inherited losses) were within the regulatory enriched intronic parts of *ALCAM* and *GRM5* respectively, both involved in major neuronal processes. We also detected a 21 kb maternally inherited X-chromosomal gain in a boy, overlapping the non-coding last exon of the gene *SYAP1* and strong regulatory elements in cis. Furthermore, we found a 44 kb loss affecting strong regulatory elements near the *DAAM1* and *DACT1* involved in the Wnt signaling pathway which co-segregated with the phenotype in a family with three affected members. Of note, in the latter two cases, whole exome sequencing did not reveal any pathogenic variant. Accordingly, we were able to illustrate a genome-wide overview of rare non-coding CNVs in patients with NDDs as well as non-coding CNVs possibly contributing to the phenotypes of 4 (3.3%) of the patients.

**P08.67****Molecular characterization of NRXN1 deletions from 19,263 clinical microarray cases identifies exons important for neurodevelopmental disease expression**

*C. Lowther<sup>1</sup>, M. Speeval<sup>2</sup>, C. Armour<sup>3</sup>, E. Goh<sup>2</sup>, G. Graham<sup>4</sup>, C. Li<sup>5</sup>, S. Zeesman<sup>5</sup>, M. Nowaczyk<sup>6</sup>, L. Schultz<sup>6</sup>, A. Morra<sup>7</sup>, R. Nicolson<sup>8</sup>, P. Bikangaga<sup>9</sup>, D. Samdup<sup>9</sup>, M. Zaazou<sup>2</sup>, K. Boyd<sup>9</sup>, J. Jung<sup>10</sup>, V. Siu<sup>11</sup>, M. Rajguru<sup>12</sup>, S. Goobie<sup>11</sup>, M. Tarnopolsky<sup>13</sup>, C. Prasad<sup>14</sup>, P. Dick<sup>14</sup>, A. Hussain<sup>10</sup>, M. Walinga<sup>15</sup>, R. Reijenga<sup>16</sup>, M. Gazzellone<sup>17</sup>, A. Lionel<sup>17</sup>, C. Marshall<sup>17</sup>, S. Scherer<sup>17,18</sup>, D. Stavropoulos<sup>19</sup>, E. McCready<sup>20</sup>, A. Bassett<sup>1</sup>.*

<sup>1</sup>University of Toronto, Toronto, ON, Canada, <sup>2</sup>Trillium Health Partners Credit Valley Site, Toronto, ON, Canada, <sup>3</sup>Regional Genetics Program, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada, <sup>4</sup>Department of Pediatrics, University of Ottawa, Ottawa, ON, Canada, <sup>5</sup>McMaster Children's Hospital, Department of Pediatrics and Clinical Genetics Program, Hamilton, ON, Canada, <sup>6</sup>Departments of Psychiatry, Western University, London, ON, Canada, <sup>7</sup>William Osler Health Centre, Brampton, ON, Canada, <sup>8</sup>Hotel Dieu Hospital, Child Development Centre, Kingston, ON, Canada, <sup>9</sup>Bethesda Services, Thorold, ON, Canada, <sup>10</sup>London Health Sciences Centre, Children's Hospital of Western Ontario, London, ON, Canada, <sup>11</sup>Department of Pediatrics, Schulich School of Medicine and Dentistry, London, ON, Canada, <sup>12</sup>Cambridge Memorial Hospital, Cambridge, ON, Canada, <sup>13</sup>Department of Pediatrics, McMaster University, Hamilton, ON, Canada, <sup>14</sup>Grey Bruce Health Services, Owen Sound, ON, Canada, <sup>15</sup>Vanboeijen, Assen, Netherlands, <sup>16</sup>Ipse de Bruggen, Zwammerdam, Netherlands, <sup>17</sup>The Centre for Applied Genomics, Toronto, ON, Canada, <sup>18</sup>McLaughlin Centre and Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada, <sup>19</sup>Cytogenetics Laboratory, Department of Pediatric Laboratory Medicine, the Hospital for Sick Children, Toronto, ON, Canada, <sup>20</sup>Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada.

**Purpose:** The purpose of the current study was to assess the penetrance of NRXN1 deletions.

**Methods:** We compared the prevalence of NRXN1 deletions identified among 19,263 clinically referred cases to that of 15,264 controls. The burden of additional clinically relevant CNVs was used as a proxy to estimate the relative penetrance of NRXN1 deletions.

**Results:** We identified 41 (0.21%) previously unreported exonic NRXN1 deletions ascertained for DD/ID, significantly greater than in controls [OR=8.33 (95% CI 2.98-23.25), p< 0.0001]. Ten (22.7%) of these had a second clinically relevant CNV. Subjects with a deletion near the 3' end of NRXN1 were significantly more likely to have a second rare CNV than subjects with a 5' NRXN1 deletion [OR=19.30 (95% CI 3.50-147.24), p<0.0001]. The prevalence of intronic NRXN1 deletions was not statistically different between cases and controls (p=0.613). The majority (63.2%) of intronic NRXN1 deletion cases had a second rare CNV, a five-fold greater prevalence than for exonic NRXN1 deletion cases (p=0.003).

**Conclusions:** The results support the importance of exons near the 5' end of NRXN1 in the expression of neurodevelopmental disorders. Intronic NRXN1 deletions do not appear to substantially increase the risk for clinical phenotypes.

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**P08.68****Intragenic duplication of the PARK2 gene contributes to developmental delay**

*P. Dello Russo, C. Gnan, A. Franzoni, N. Passon, F. Baldan, L. Allegri, G. Damante; Azienda Ospedaliero Universitaria S.Maria della Misericordia, Udine, Italy.*

**Introduction:** Autism spectrum disorder (ASDs) are childhood neurodevelopmental disorders genetically heterogeneous, with evidence for hundreds of susceptibility loci. Recently it has been reported that CNVs within or surrounding genes involved in the ubiquitin pathways, including *UBE3A*, *PARK2*, *RFWD2* and *FBXO40*, were detected as possible cause for ASD. Furthermore *PARK2* gene was identified as candidate gene for attention-deficit/hyperactivity disorder. The precise function of this gene is unknown yet; however the parkin protein seems implicated in dopaminergic transmission.

Recently was identified a 314kb copy number gain in the long arm of chromosome 6q26 including only exon 2 of *PARK2* gene associated to a 3-month-old female with neurological problems.

We report a ten-year-old boy who showed behavior problems, language difficulties, psychomotor retardation, cognitive impairment, and no dysmorphic features.

**Materials and Methods:** Array-CGH assay was carried out (Agilent Human Genome CGH oligonucleotide array 180k, Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's instructions on a DNA sample isolated from peripheral blood cells.

Quantitative PCR was performed using 7300 Real Time PCR System (Applied BioSystems, Foster City, CA, USA) on the samples of our proband and his parents to determine the inheritance of chromosomal aberration.

**Results:** Array-CGH revealed a duplication of 231kb in the chromosome 6q26 which only included two exons (exon 3 and 4) of *PARK2* gene. He had no other pathogenetic CNV. Real Time PCR revealed that the rearrangement was paternally inherited.

**Conclusion:** Our patient supports the positive association between *PARK2* gene damages and neurocognitive and developmental anomalies.

**P08.69****Effect of intranasal insulin on development in Phelan-McDermid syndrome: a randomized, double-blind, placebo-controlled trial**

*R. J. Zwanenburg<sup>1</sup>, G. Bocca<sup>2</sup>, S. A. J. Ruiter<sup>3</sup>, J. H. Dillingh<sup>4</sup>, B. C. T. Flapper<sup>2</sup>, E. R. van den Heuvel<sup>5</sup>, C. M. A. van Ravenswaaij-Arts<sup>6</sup>;*

<sup>1</sup>Department of Genetics, UMCG, Groningen, Netherlands, <sup>2</sup>Department of Paediatrics, UMCG, Groningen, Netherlands, <sup>3</sup>Centre of Expertise for Child Development Care and Research, Groningen, Netherlands, <sup>4</sup>Department of Clinical Pharmacy and Pharmacology, UMCG, Groningen, Netherlands, <sup>5</sup>Department of Mathematics and Computer Science, Eindhoven University of Technology, Eindhoven, Netherlands.

**Introduction:** Phelan-McDermid (PMS) or 22q13.3 deletion syndrome is a rare neurodevelopmental disorder. Clinical features include moderate to severe intellectual disability and behavioural problems in the autism spectrum. Other researchers had observed a beneficial effect of intranasal insulin on development and behaviour in a pilot study in six children with PMS. To validate this effect, we conducted a randomized, double-blind, placebo-controlled clinical trial using a stepped-wedge design.

**Material and methods:** Twentyfive children aged 1 to 16 years with a 22q13.3 deletion including the SHANK3-gene participated in the clinical trial for a period of 18 months. Starting 6 months before the trial, children were systematically assessed for development and behaviour every 6 months. The 2nd, 3rd and 4th assessments were followed by daily nose sprays containing either insulin or placebo for a 6-month period. A 5th assessment was done directly after the end of the trial.

**Results:** Intranasal insulin did not cause serious adverse events. It increased the level of developmental functioning by 0.4-1.4 months per 6-month period that, while not statistically significant in this small group, was considered clinically relevant. A stronger, significant effect was observed in children older than 3 years, who usually show a decrease of developmental growth. **Conclusions:** Although the application of intranasal insulin is a promising therapeutic approach, clinical trials in larger study populations are required to prove the therapeutic effect of intranasal insulin in PMS.

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Contact: 22q13@umcg.nl

**P08.70****Diagnostic flow-chart to improve the detection rate and to uncover the genetic heterogeneity in Pitt-Hopkins syndrome: experience on 260 subjects**

*M. Zollino, S. Ricciardi, S. Frangella, D. Orteschi, M. Murdolo, P. Doronzio, S. Lattante, G. Marangi;*

*Institute of Genomic Medicine, Università Cattolica del Sacro Cuore, Roma, Italy.*

Pitt-Hopkins syndrome (PTHS) is defined by the association of severe intellectual disability, a typical facial gestalt and additional features, including breathing abnormalities. It is caused by haploinsufficiency of the *TCF4* gene. The PTHS phenotype overlaps with different conditions, including mainly Angelman, but also Mowat-Wilson, Christianson and Rett syndromes.

A total of 260 subjects were referred because of clinical suspicion of PTHS. The genetic diagnosis was obtained in 40/260 (15%) as follows: *TCF4* intragenic variants (30); large 18q21 deletions (2); partial *TCF4* deletion (1); balanced de novo translocations (2); *MECP2* variants (2); *UBE3A* variant (1); *ZEB2* variants (2).

All patients were first distinguished into two categories, depending on clinical presentation. Group A included 125 subjects who all had severe ID with nearly absent speech and high clinical score for PTHS; group B included the remaining 135 subjects with mild to moderate ID and low clinical score for PTHS.

All patients with a proven genomic mutation were in group A, increasing test sensitivity from 15% to 32%.

A clinically driven flowchart is suggested in PTHS phenotype, including step by step the following tests: 1) *TCF4* analysis; 2) array-CGH; 3) conventional cytogenetics; 4) NGS gene panel analysis (*TCF4*, *CNTNAP2*, *NRXN1*, *UBE3A*, *SLC9A6*, *MECP2*, *CDKL5*, *FOXP1*, *MEF2C*, *ZEB2*, *ATRX*); 5) whole exome se-

quencing (WES). Variants detected by WES are presented separately, since a limited number of patients has been analyzed. Nosological implications are discussed. Supported by Telethon grant nr: GEP 14089.

## P08.71

### Two unrelated cases with rare mosaic deletions affecting TCF4 gene, exhibiting different phenotypes

*A. Alexandrou<sup>1</sup>, G. A. Tanteles<sup>2,3</sup>, I. Papaevripidou<sup>1</sup>, A. Keton<sup>1</sup>, N. Salameh<sup>1</sup>, P. Evangelidou<sup>1</sup>, C. Sismani<sup>1</sup>, V. Christodou-Anastasiadou<sup>2,3</sup>,*

<sup>1</sup>*Cytogenetics and Genomics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>Clinical Genetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>3</sup>Archbishop Makarios III Medical Centre, Nicosia, Cyprus.*

Pitt-Hopkins syndrome (PTHS) is a rare neurodevelopmental disorder characterized by a distinctive phenotype. PTHS phenotype is caused by mutations or variable size deletions involving TCF4 gene (18q21) implying haploinsufficiency of this gene. Mosaic structural rearrangements are extremely rare events and pose great challenges for interpretation. There is very limited literature on mosaic deletions affecting TCF4 gene in which patients are described to manifest the full spectrum of the disorder. Herein, we report two unrelated individuals bearing deletions in a low mosaic state.

The first case is the non-affected father of a patient with a PTHS spectrum phenotype. A deletion of 263.4 kb was identified in the patient removing exons 4-9 of TCF4 gene. The deletion was inherited from the father who carries the deletion in a mosaic state (~20%). This is the first PTHS case reported in the literature of a deletion affecting TCF4 inherited from a clinically unaffected parent.

The second case involves a patient with a phenotype consistent with PTHS. Array-CGH revealed a very low mosaic (15%) 18q21.2q21.33 deletion of 10.17Mb in size harbouring the TCF4 gene which was confirmed by FISH analysis (11%). Family studies are ongoing.

Our findings and review of the literature support that deletions affecting TCF4 independent of their size appear to have no significant effect on the severity of the syndrome. However, it is also demonstrated that mosaic 18q21 deletions can have a normal phenotype which contradicts previous observations that mosaic status of such deletions cause similar phenotypes.

## P08.72

### Characterization of patient with *de novo* frameshift PUF60 mutation suggests that disruption of this gene is sufficient for most symptoms of Verheij syndrome

*M. Hančárová<sup>1</sup>, M. Havlovicová<sup>1</sup>, D. Prchalová<sup>1</sup>, V. Stránecký<sup>2</sup>, Z. Sedláček<sup>1</sup>;*

<sup>1</sup>*Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, <sup>2</sup>Institute of Inherited Metabolic Disorders, Charles University 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic.*

Verheij syndrome (OMIM 615583) is associated with rare *de novo* 8q24.3 microdeletions. Patients show developmental delay, short stature, microcephaly, coloboma, and craniofacial, skeletal, cardiac and renal defects. Initially two patients were described with deletions of 15-24 Mb, later followed by five patients with much shorter deletions, the smallest encompassing only three genes, SCRIB, PUF60 and NRBP2. A *de novo* loss-of-function missense PUF60 mutation was also found in a similar patient who however lacked coloboma and renal abnormalities. These observations and knockdown of PUF60 and/or SCRIB in zebrafish suggested that the two genes influenced different symptoms, together exacerbating the phenotype of the syndrome. We report a 16-year-old boy with autism, moderate intellectual disability, speech delay, growth retardation, short stature, microcephaly, cardiac and renal defects, skeletal anomalies and a facies with bitemporal narrowing, thick eyebrows, long philtrum, broad nasal root and thin upper lip. Karyotyping and microarray analysis yielded no findings. Whole exome sequencing of the family trio identified a private heterozygous *de novo* deletion of four nucleotides in PUF60 (NM\_078480.2:c.407\_410delCTCA,p.I136Tfs\*31). The patient showed no eye symptoms but no focused ophthalmological examination has been performed yet. The presence of severe renal hypoplasia in the patient and his overall similarity to the typical picture of the Verheij syndrome suggest that also the renal symptoms can be attributed solely to defects of PUF60 which may be of key importance for the syndrome. The case illustrates the strength of exome sequencing in deciphering the genetic basis of diseases.

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## P08.73

### First case of concurrent RAI1 mutation and ANKRD11 partial deletion in a girl with features of Smith Magenis and KBG syndromes

*I. Bestetti<sup>1,2</sup>, C. Picinelli<sup>1</sup>, M. Crippa<sup>1</sup>, M. Mencarelli<sup>3</sup>, L. Larizza<sup>1</sup>, D. Milani<sup>4</sup>, P. Finelli<sup>1,2</sup>;*

<sup>1</sup>*Laboratory of Medical Cytogenetics and Molecular Genetics, IRCCS Istituto Auxologico Italiano, Milano, Italy, <sup>2</sup>Department of Medical Biotechnology and Translational Medicine, University of Milan, Milano, Italy, <sup>3</sup>Molecular Biology Laboratory, IRCCS Istituto Auxologico Italiano, Milano, Italy, <sup>4</sup>Medical Genetic Unit, Pediatric Highly Intensive Care, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Smith Magenis (SMS) and KBG syndromes are rare autosomal dominant disorders characterized by distinctive facial features and intellectual disability (ID), caused by RAI1 and ANKRD11 haploinsufficiency, respectively. Here we describe a 46,XX girl aged 8 years, who shows mild to moderate facial dysmorphisms, ID, and an autism spectrum disorder. Upon exclusion of 17p11.2 SMS locus deletion by previous genetic investigation, RAI1 mutational screening identified an unreported heterozygous missense mutation (p.G1036R) inherited from the healthy father who shares with his daughter the facial aspect only. MLPA and RT-qPCR analyses on RAI1 revealed a wild type condition, whereas high resolution array-CGH analysis disclosed a rare *de novo* deletion at 16q24.3, never reported in healthy subjects. The deletion affects the last two exons and the 3'UTR of ANKRD11. RT-qPCR performed on the KBG gene revealed the simultaneous existence of wild type and truncated mRNA, each present at 50% of the total amount detected in healthy controls.

Based on these findings and considering that ANKRD11 mutations have been reported in a few clinically borderline Cornelia de Lange (CdLS) patients, a clinical revaluation of the proband was performed. Neither microcephaly and developmental delay, generally associated to CdLS, nor macrodontia and short stature, hallmarks of the classic KBG phenotype, were observed. However, the proband shows a composite phenotype mixing up KBG and SMS signs. This peculiar clinical picture might result from the combined effect of RAI1 mutation and ANKRD11 *de novo* deletion, which does not likely imply gene haploinsufficiency, as in classical SMS and KBG syndromes.

## P08.74

### Copy number variation in RBFOX1 and DOCK8: Neurodevelopmental disorders or benign variants?

*S. Alaba<sup>1,2</sup>, K. E. Hermetz<sup>2</sup>, M. Rudd<sup>2</sup>;*

<sup>1</sup>*Leeds Genetics Lab, Leeds, United Kingdom, <sup>2</sup>Emory Genetics Laboratory, Atlanta, GA, United States.*

Deletions and duplications involving the genes RBFOX1 and DOCK8 have been implicated in intellectual disability (ID) and autism spectrum disorders (ASD). However, the clinical significance of these CNVs is controversial. We analysed 14 RBFOX1 deletions (20-309 kb), six RBFOX1 duplications (34 kb to 1.34 Mb), and eight DOCK8 deletions (22-227 kb) from unrelated individuals. Our analysis revealed that five DOCK8 deletions were terminal and three were interstitial. Two deletions lie within intron 1, two include only exon 1, and four include multiple exons. Thirteen of the RBFOX1 deletions lie within a single intron; deletion of intron 3 and intron 4 are the most common. Though the clinical information provided is limited, seven individuals with RBFOX1 deletions have a diagnosis of ASD. The clinical significance of duplications is difficult to interpret since intragenic gains can lead to loss-of-function, whereas whole gene duplication can lead to triplosensitivity. Half of the RBFOX1 duplications have one breakpoint upstream of the gene and one breakpoint in intron 2, which is predicted to maintain the reading frame if the duplications are in direct tandem orientation. One RBFOX1 duplication lies entirely within intron 3 and two include only exon 4, which could lead to an out-of-frame transcript. Our analysis sheds light on the genomic structures of small CNVs implicated in neurodevelopmental disorders. Only two CNVs had identical breakpoints, suggesting diversity in RBFOX1 and DOCK8 rearrangements rather than a few common variants. However, the clinical significance of these CNV remains unclear since all CNVs we included were inherited.

**P08.75****Molecular diagnosis improvement in patients with Rett Syndrome using Next-Generation Sequencing.**H. Maortua<sup>1,2,3</sup>, A. De la Hoz<sup>2,3</sup>, C. Martínez-Bouzas<sup>1,2,3</sup>, M. I. Tejada<sup>1,2,3</sup>;<sup>1</sup>Genetic Service, Cruces University Hospital, Barakaldo-Bizkaia, Spain, <sup>2</sup>BioCruces Health Research Institute, Cruces University Hospital, Barakaldo-Bizkaia, Spain, <sup>3</sup>Clinical group affiliated with the Centre for Biomedical Research on Rare Disease (CIBERER), Madrid, Spain.

Introduction: Rett syndrome (RTT) is a severe neurodevelopmental disorder characterized by intellectual disability, regression of development and progressive microcephaly. RTT is mainly caused by mutations in MECP2; although mutations in other genes (CDKL5 and FOXG1) are also found. However, a number of patients who show a clinical diagnosis of RTT have no mutations in these genes. Recently, Next Generation Sequencing technology (NGS) has changed genetic diagnosis because of its ability to detect rare, de novo mutations.

Material and Methods: We designed a NGS panel with 21 genes (ARX, BDNF, CDKL5, FOXG1, FOXP1, FOXP2, GABRD, HERC2, JMJD1C, KCNQ2, MECP2, MEF2C, MFSD8, PLP1, SCN2A, SHANK3, STXBP1, TBR1, TCF4, UBE3A and WDR45). The panel consists of 11874 amplicons (196.569 Kb), with 98.57% target coverage. Target enrichment was performed by HaloplexHS. Libraries were sequenced on an Ion Torrent PGM platform. SureCall software was used for raw data alignment and variant annotations. Torrent SuiteTM and Ion ReporterTM software were then used for variant analysis.

Results: Our custom panel has been validated detecting all the known molecular changes (table below). Furthermore, new variations have been found which are under study.

Conclusion: This custom panel is a powerful and cost-effective tool to diagnose complex disorders with genetic heterogeneity such as RTT syndrome. This work has been funded by Fundació Agrupació.

Gene	Variations
MECP2	16 loss-of-function
MECP2	4 missense
MECP2	4 polymorphism
CDKL5	1 loss-of-function
CDKL5	1 deletion
CDKL5	1 unknown variation
ARX	1 missense
ATRX	1 missense
UBE3A	2 loss-of-function
UBE3A	1 missense

**P08.76****First familial Rubinstein-Taybi syndrome case associated with a novel EP300 mutation**M. López<sup>1</sup>, P. de Castro Castro<sup>2</sup>, V. Seidel<sup>2</sup>, E. Domínguez-Garrido<sup>1</sup>;<sup>1</sup>Fundación Rioja Salud, Logroño, Spain, <sup>2</sup>Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Introduction: RSTS (OMIM RSTS 1, #180849, RSTS 2, #613684) is a rare (1:125000) autosomal dominant neurodevelopmental disorder characterized by broad thumbs and halluces. The vast majority of cases occur sporadically due to de novo heterozygous mutations. RSTS is caused in 50-60% of cases by mutations in the CREBBP gene and by EP300 gene mutations in 5-8%.

Subjects and methods: A 9 year-old female patient was referred from Neuropediatrics for suspected RSTS. DNA was obtained from blood of the patient and her mother. CREBBP and EP300 MLPA, panel based-NGS of CREBBP and EP300 genes and Sanger sequencing confirmation was carried out. Sequence changes were compared to the mother.

Results: The patient and her mother presented with mild learning difficulties, short stature and microcephaly. The mother had broad thumbs, whereas the child had normal thumbs and halluces. A novel heterozygous deletion in exon 31 of EP300 gene (c. 7219\_7222del) was found in both the proband and her mother, indicating that the mutation is inherited. This is a clearly pathogenic frameshift mutation (p.S2407fs).

Conclusion: This is the first report of an inherited EP300 mutation in RSTS worldwide, and the first familial RSTS case in Spain, demonstrating that transmission is extremely rare. Since most individuals with RSTS due to EP300 mutation are mildly affected, this entity is likely underdiagnosed, thus special attention should be paid in them.

**P08.77****Rubinstein-Taybi syndrome type 2: Report of 9 new cases that extend the phenotypic and genotypic spectrum**M. J. Hamilton<sup>1,2,3</sup>, R. Newbury-Ecob<sup>4</sup>, M. Holder-Espinasse<sup>5</sup>, J. A. Hurst<sup>6</sup>, E. Clement<sup>6</sup>, W. Reardon<sup>7</sup>, S. Joss<sup>2</sup>, E. Hobson<sup>8</sup>, M. Blyth<sup>9</sup>, M. Al-Shehhi<sup>7</sup>, S. Lynch<sup>9</sup>, M. Suri<sup>3</sup>;<sup>1</sup>University of Glasgow, Glasgow, United Kingdom, <sup>2</sup>West of Scotland Clinical Genetics Service, Glasgow, United Kingdom, <sup>3</sup>Department of Clinical Genetics, Nottingham City Hospital, Nottingham, United Kingdom, <sup>4</sup>Department of Clinical Genetics, University Hospitals Bristol, Bristol, United Kingdom, <sup>5</sup>Clinical Genetics Service, Guy's and St Thomas' Hospital, London, United Kingdom, <sup>6</sup>Clinical Genetics Unit, Great Ormond Street Hospital for Children, London, United Kingdom, <sup>7</sup>Department of Clinical Genetics, Our Lady's Children's Hospital, Dublin, Ireland, <sup>8</sup>Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds, United Kingdom, <sup>9</sup>ACORD, University College Dublin, Dublin, Ireland.

BACKGROUND: Rubinstein-Taybi syndrome is an autosomal dominant neurodevelopmental disorder characterised by growth deficiency, broad thumbs and great toes, intellectual disability and characteristic craniofacial appearance. Mutations in CREBBP account for around 55% of cases, with a further 8% attributed to the paralogous gene EP300. Comparatively few reports exist describing the phenotype of Rubinstein-Taybi due to EP300 mutations. METHODS: Clinical and genetic data were gathered from 9 patients from the UK and Ireland with pathogenic EP300 mutations, identified either by targeted testing or exome sequencing. RESULTS: All patients had mild or moderate intellectual impairment. Behavioural or social difficulties were noted in eight, including three with autistic spectrum disorders. Typical dysmorphic features of Rubinstein-Taybi were only variably present. Additional observations include maternal pre-eclampsia (2/9), syndactyly (3/9), delayed bone age (2/9), feeding or swallowing issues (2/9), scoliosis (2/9) and hypermobility of the elbow (2/9). Six patients had truncating mutations in EP300, with pathogenic missense mutations identified in the remaining three. DISCUSSION: The findings support previous observations that microcephaly, maternal pre-eclampsia, mild growth restriction and a mild to moderate intellectual disability are key pointers to the diagnosis of EP300-related Rubinstein-Taybi syndrome. Variability in the presence of typical facial features of Rubinstein-Taybi further highlights clinical heterogeneity, particularly among patients identified by exome sequencing. Features that overlap with Floating-Harbor syndrome including craniofacial dysmorphism and delayed osseous maturation were observed in 3 patients. Previous reports have only described mutations predicted to cause haploinsufficiency of EP300. This cohort includes the first described pathogenic missense mutations in EP300.

**P08.78****Abnormal primary dentition : a clue for the diagnosis of the SATB2-associated syndrome**M. Rio<sup>1,2</sup>, G. García<sup>1,2</sup>, J. Bonnefont<sup>1,2</sup>, J. Amiel<sup>1,2</sup>, V. Pingault<sup>1,2</sup>, S. Hanein<sup>2</sup>, A. Munnoch<sup>1,2</sup>;<sup>1</sup>Service de génétique, Hôpital Necker-Enfants Malades, APHP, Paris, France, <sup>2</sup>INSERM 1163 Institut IMAGINE Université Paris Descartes-Sorbonne Paris Cité, Paris, France.

The SATB2-associated syndrome (SAS) has been recently proposed as a new clinically recognizable syndrome that results from deleterious alterations of the SATB2 gene in humans. We report the clinical characterization of 5 patients in whom targeted high-throughput sequencing for the diagnosis of intellectual disability or neurocristopathies identified a de novo mutation in the SATB2 gene for three patients and a de novo intragenic deletion for two patients. Age at diagnosis was 5 years (2/5), 7 years (2/5), 8 years (1/5). The 5 patients shared the characteristic features of the SAS: intellectual disability with limited speech development (5/5), cleft (1/5) or high-arched palate (4/5), and dental abnormalities (5/5). 4 patients presented one fracture and osteopenia was seen in two of the patients, confirming that this finding could be added to the list of distinctive findings. All patients had dental abnormalities with macrodontia of primary and permanent teeth, irregular shape of the teeth, dental crowding. Interestingly, anomaly of the primary teeth is a consistent finding detected in the 5 patients. This feature should be a good handle for early diagnosis of SAS and we therefore suggest adding this feature to the list of distinctive features of SAS.

**P08.79****Severe presentation of primary microcephaly (Seckel-like syndrome) in a patient born from an incestuous relationship**

I. Sudholt, K. Komlosi, S. Diederich, D. L. Fend-Guella, U. Zechner, S. Schweiger, O. Bartsch;

Institute of Human Genetics, University Medical Center of the Johannes Gutenberg University, Mainz, Germany.

Seckel syndrome is a genetically heterogenous rare recessive disorder with growth retardation, microcephaly, intellectual disability (ID) and a bird-

headed' appearance. The differential diagnosis includes autosomal recessive primary microcephaly (MCPH) without additional malformations or neurologic findings.

We report a 30-year-old woman with profound ID, absent speech, stereotypic behavior, diplegia, proportionate growth deficiency (-5 SD), severe primary microcephaly (-10,5 SD) and dysmorphic features including a sloping forehead, prominent large nose, small chin, dorsally rotated ears, short and broad fingers, and small feet. She was a product of an incestuous abuse between father and daughter. In the first 30 years she had the clinical diagnosis of Seckel syndrome with no identified genetic cause. We used panel diagnostics for over 1200 brain related genes (MPIMG-1-Test) on an Illumina MiSeq system and a modified Medical Resequencing Analysis Pipeline for variant calling.

3/40 variants fitted an autosomal recessive model with only STIL associated with the phenotype. A novel homozygous likely pathogenic sequence variant STIL c.3377A>G (p.Tyr1126Cys) was found. Segregation analysis confirmed heterozygosity in the mother. The father (=grandfather) was deceased. Homozygous STIL mutations can cause MCPH7 (OMIM #612703) with non-syndromic primary microcephaly and moderate ID, however, single cases also showed severe ID, short stature, and holoprosencephaly. Possibly additional yet unidentified recessive traits in our patient might have contributed to a more complex phenotype of classical MCPH7. Our case demonstrates the overlap between syndromic and non-syndromic forms, the close connection to Seckel syndromes and the clinical utility of NGS in the diagnosis of atypical presentations.

#### P08.80

#### The identification of a 94,5 kb deletion in Xq24 encompassing the SLC25A5 gene confirms its pathogenic role in neurodevelopmental disorders

A. Zonta<sup>1</sup>, G. Zucchetti<sup>1</sup>, I. Fusco<sup>1</sup>, S. Mellone<sup>1</sup>, C. Peruzzi<sup>2</sup>, A. Guala<sup>3</sup>, M. Giordano<sup>1</sup>;

<sup>1</sup>Human Genetics Laboratory, Dept. of Health Sciences, Piemonte Orientale University, Novara, Italy, <sup>2</sup>Child Neurology Unit, Maggiore della Carità Hospital, Novara, Italy,

<sup>3</sup>Pediatric Unit, Castelli Hospital, Verbania, Italy.

We report on a 16-year-old boy referred for learning disability, deficit in attention and social competence, difficulties in daily activities organization, impulsive and repetitive behaviour, clumsiness and fatigue. The clinical examination reported bilateral epicanthus, left palpebral ptosis and valgus knees. An EEG showed diffuse abnormalities with a poorly organized background. EMG, NCV and CK were normal. His maternal uncle is affected with intellectual disability and epilepsy. His parents are healthy and non consanguineous.

Array-CGH[60K] identified a 94,5 kb deletion at Xq24 (chrX:118.579.289-118.673.787) encompassing the 3' end of the SLC25A43 gene, the SLC25A5 gene and the 3' end of the CXorf56 gene. The same deletion has been found in the proband's mother, maternal grandmother, maternal uncle with intellectual disability and seizures and was not found in another healthy maternal uncle.

Overlapping microdeletions at Xq24 have been found in patients with non syndromic intellectual disability or borderline IQ scores. The smallest region of overlap encompassed the SLC25A43 and SLC25A5 genes, members of the mitochondrial carrier subfamily of solute carrier (SLC) protein genes. Biallelic mutations of genes belonging to the SLC25 family cause a wide range of metabolic disorders with CNS involvement. As a deletion of the SLC25A43 gene has been subsequently found in a healthy subject and the expression of this gene in the brain is low, a pathogenic role for the SLC25A5 has been suggested.

The deletion identified in our patient is the smallest deletion reported so far encompassing the SLC25A5 gene and confirms that its deficiency can cause neurodevelopmental disorders.

#### P08.81

#### MBD5 molecular screening on Smith Magenis-like Syndrome patients without the typical 17p11.2 deletion

M. Sciarillo<sup>1,2</sup>, C. Picinelli<sup>1</sup>, I. Bestetti<sup>1,2</sup>, A. Sironi<sup>1</sup>, N. Kurtas<sup>1</sup>, E. Ponti<sup>3</sup>, A. Mihalich<sup>3</sup>, L. Spaccini<sup>4</sup>, M. Bedeschi<sup>5</sup>, C. Pantaleoni<sup>6</sup>, S. Maitz<sup>7</sup>, A. Selicorni<sup>7</sup>, D. Milani<sup>8</sup>, L. Larizza<sup>1</sup>, P. Finelli<sup>1,2</sup>;

<sup>1</sup>Laboratory of Medical Cytogenetic and Molecular Genetics, IRCCS Istituto Auxologico Italiano, Milano, Italy, <sup>2</sup>Department of Medical Biotechnology and Translational Medicine, University of Milan, Milano, Italy, <sup>3</sup>Molecular Biology Laboratory, IRCCS Istituto Auxologico Italiano, Milano, Italy, <sup>4</sup>Medical Genetics Unit, Buzzi Children's Hospital, Milano, Italy, <sup>5</sup>Medical Genetic Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy, <sup>6</sup>Infantile Neuropsychiatry, U.O. Developmental Neurology, IRCCS Istituto Neurologico „Carlo Besta“, Milano, Italy, <sup>7</sup>U.O.S Clinical Genetics and Pediatrics, MBBM Foundation San Gerardo Hospital, Monza, Italy, <sup>8</sup>Medical Genetic Unit, Pediatric Highly Intensive Care, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.

Smith Magenis Syndrome (SMS) is a complex heterogeneous disorder, caused by RAI1 haploinsufficiency and triggered by 17p11.2 deletion or RAI1 gene mutation. Only 50% of patients with a clinical suspicion of SMS bear the known defects, making its diagnosis challenging and suggesting that other loci may underlie SMS-like phenotypes.

Notably 2q23.1 deletion and brachydactyly mental retardation (BDMR) syndromes, caused by MBD5 and HDAC4 haploinsufficiency respectively, are two SMS phenotypical and molecular overlapping syndromes as the affected patients seem to show a reduced RAI1 transcript levels, implying that MBD5/HDAC4 regulate RAI1.

Here we describe a cohort of 30 SMS-like patients without 17p11.2 deletion, and RAI1 microdeletion or mutation. Array-CGH analysis revealed a 29 kb deletion at 2q23.1 encompassing exons 1 and 2 of MBD5 in one patient, while MBD5 mutational screening identified in a second case an heterozygous missense mutation likely pathogenetic, p.A857T. Unexpectedly the deletion was found in a mosaic condition in the healthy father of the first patient, while the missense mutation, previously described in a child with mental retardation, was maternally inherited. The subsequent RT-qPCR showed a MBD5 transcript downregulation in the patient with 2q23.1 deletion, normal RAI1 transcript levels in all the patients and unexpectedly in the SMS-like patient with MBD5 haploinsufficiency too.

Our findings corroborate the hypothesis that other loci can be causative of SMS-like phenotypes and for this reason we are setting up a flow chart aiming at improve the molecular diagnosis in SMS-like patients and shed light on pathogenetic mechanisms.

#### P08.82

#### Identification of a RAI1-associated disease network

A. Reymond<sup>1</sup>, M. Loviglio<sup>1</sup>, C. R. Beck<sup>2</sup>, T. Harel<sup>2</sup>, W. Bi<sup>2</sup>, M. Leleu<sup>3</sup>, N. Guex<sup>1,4</sup>, A. Niknejad<sup>4</sup>, E. S. Chen<sup>2</sup>, E. S. Chen<sup>2</sup>, S. Gu<sup>2</sup>, J. White<sup>2</sup>, I. Crespo<sup>4</sup>, J. Yan<sup>2</sup>, W. Charng<sup>2</sup>, C. A. Shaw<sup>2</sup>, Z. Coban-Akdemir<sup>2</sup>, J. Rougemont<sup>2</sup>, I. Xenarios<sup>1,4</sup>, J. R. Lupski<sup>2</sup>;

<sup>1</sup>Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland,

<sup>2</sup>Baylor College of Medicine, Houston, TX, United States, <sup>3</sup>Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, <sup>4</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Smith-Magenis syndrome (SMS) is a developmental disability/multiple congenital anomaly disorder resulting from haploinsufficiency of RAI1. We investigated a cohort of 149 individuals presenting the constellation of SMS features, and focused on 15 individuals showing neither hemizygosity in the SMS critical region nor variants in RAI1. Using whole-exome sequencing we identified potentially deleterious variants in eight patients. These variants affect KMT2D, ZEB2, MAP2K2, GLDC, CASK, MECP2, KDM5C and POGZ, known to be associated with Kabuki 1, Mowat-Wilson, cardiofaciocutaneous, glycine encephalopathy, mental retardation and microcephaly with pontine and cerebellar hypoplasia, X-linked mental retardation 13, X-linked mental retardation Claes-Jensen type and a recently described intellectual disability syndrome, respectively. Analyses of coexpression networks and biomedical text mining suggest that these pathologies and SMS are part of the same disease network. Further support for this hypothesis comes from transcriptome profiling of 10.5 dpc embryos that shows that the expression levels of both ZEB2 and MAP2K2 are perturbed in Rai1-/- mice. Chromatin conformation capture revealed contacts between RAI1 and the ZEB2 and GLDC flanking loci, as well as between RAI1 and human orthologs of the genes that show perturbed expression in a Rai1-/- mouse model, in particular genes mapping to MMU11. This finding possibly explains the enrichment of MMU11-mapping genes within genes differentially expressed in Rai1-/- mouse embryos. These holistic studies of RAI1 and its interactions allow insight into SMS and other disorders associated with intellectual disability and behavioral abnormalities, demonstrating the utility of a comprehensive genomic approach even in the diagnosis of distinctive disorders.

**P08.83****Exome Sequencing Combined with Linkage Analysis Identifies a Novel Gene Associated with a Syndromic Form of Intellectual Disability**Y. Kesim<sup>1</sup>, M. Calik<sup>2</sup>, F. N. Tunçer<sup>1</sup>, G. Altıokka Uzun<sup>3</sup>, A. İscan<sup>4</sup>, U. Ozbek<sup>1</sup>, S. Uğur Iseri<sup>1</sup>,<sup>1</sup>Institute for Experimental Medicine, Genetics Department, Istanbul, Turkey, <sup>2</sup>Harran University, Faculty of Medicine, Department of Pediatric Neurology, Sanliurfa, Turkey,<sup>3</sup>Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, Istanbul, Turkey, <sup>4</sup>Bezmialem Vakif University, Faculty of Medicine, Department of Pediatric Neurology, Istanbul, Turkey.

**Introduction:** Syndromic intellectual disability (ID) comprises a group of clinically and genetically heterogeneous conditions, in which intellectual deficits are associated with other medical and behavioral signs and symptoms. Herein, we present genetic analyses leading to identification of a novel gene associated with syndromic ID in a consanguineous family.

**Materials and Methods:** All family members including two affected siblings were SNP genotyped in order both to perform genome wide copy number variation (CNV) profiling and linkage analysis. This strategy was accompanied by exome sequencing in the affected sib pairs.

**Results:** CNV analysis did not detect common pathogenic CNV events in the two affected sibs. SNP derived linkage analysis was overlapped with exome variants, which enabled us to investigate exome variants only in linkage intervals. This collective effort led us to identify a new substitution in a novel gene encoding a member of alpha/beta hydrolase superfamily on chromosome 20. Upon this finding, the first-degree cousin of the affected sib pair having a similar phenotype was also recruited to the study. The variant was also shown to be segregating with the disorder in this new branch of the family. Pathogenicity of the variant is supported further by *in silico* tools and its absence in public databases and apparently healthy 300 controls from Turkey.

**Conclusions:** Combined analysis of linkage and exome sequencing has proved to be a powerful approach proved to identify defective genes in consanguineous pedigrees. This study was supported by the grants of TUBITAK (113S331) and Istanbul Development Agency (TR10/15/YNK/0093).

**P08.85****A new patient with Treacher Collins syndrome and intellectual disability due to a small deletion of TOCF1 and CAMK2A genes**I. López-Expósito<sup>1,2,3</sup>, J. Bafalliu<sup>1,2,3</sup>, M. Ballesta-Martínez<sup>4,2,3</sup>, A. Vera-Carbonell<sup>1,2,3</sup>, V.López-González<sup>4,2,3</sup>, G. Soler-Sánchez<sup>1,3</sup>, M. Sánchez-Soler<sup>4</sup>, E. Guillén-Navarro<sup>5,2,3</sup>,<sup>1</sup>Centro de Bioquímica y Genética Clínica. Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER). Instituto de Salud Carlos III, Madrid, Spain, <sup>3</sup>Instituto Murciano de Investigación Biosanitaria (IMIB), Murcia, Spain, <sup>4</sup>Sección de Genética Médica. Servicio de Pediatría. Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain, <sup>5</sup>Consejería de Sanidad, Murcia, Spain.

The Treacher Collins syndrome-1 (TCS1, OMIM 154500) is an autosomal dominant disorder of craniofacial development, caused by heterozygous mutation in the TOCF1 gene on chromosome 5q32, rarely associated to intellectual disability (ID).

Recently, two large deletions (262kb and 1Mb) encompassing TOCF1 have been described in two patients with typical features of TCS and ID. The minimal critical region also involved the CDX1, SLC6A7, CAMK2A, ARSI and CD74 genes. Authors suggested that CAMK2A could be responsible for the ID, although a combinatorial effect with the other deleted genes could not be ruled out.

We report on a new patient with TCS1 and ID, with a smaller deletion (ranging from 150kb to 180 Kb) that only included TOCF1, ARSI and CAMK2 genes. The girl was referred for genetic evaluation at four months of age, due to abnormal craniofacial development. MLPA of TOCF1 showed a complete deletion of the gene. At the last evaluation at the age of 10 years, her facial features fit the diagnosis of TCS and she also presented behavioral disorders and intellectual disability. CGH array was performed confirming that ARSI and CAMK2A were also deleted. The function of the ARSI gene is not related with the phenotype of the patient, but CAMK2A can play a role in the neuronal development.

In conclusion, our patient presents a novel small deletion that narrows down the minimal critical region for a contiguous genetic disorder with TSC and ID, and strongly supports the hypothesis that haploinsufficiency of CAMK2A gene is responsible for the ID.

**P08.86****Clinical description of two cases of Trichothiodystrophy 4 (OMIM#234050) due to homozygous mutation in MPLKIP in two unrelated families from the same geographical area, suggesting a common founder**M. Ballesta-Martínez<sup>1</sup>, V. López-González<sup>1</sup>, M. Sánchez-Soler<sup>1</sup>, L. Rodríguez-Peña<sup>1</sup>, B. Rodríguez-Santiago<sup>2</sup>, T. Martínez-Menchón<sup>3</sup>, J. Ferrando-Barbera<sup>4</sup>, L. Armengol-Dulcet<sup>2</sup>, E. Guillén-Navarro<sup>5</sup>;<sup>1</sup>Sección de Genética Médica. Servicio de Pediatría. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, <sup>2</sup>qGenomics, Barcelona, Spain, <sup>3</sup>Servicio de Dermatología. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, <sup>4</sup>Servicio de Dermatología. Hospital Clínico de Barcelona, Barcelona, Spain, <sup>5</sup>Consejería de Sanidad, Murcia, Spain.

Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterized by short, brittle hair with low-sulphur content. TTD patients display a wide variety of clinical features, including cutaneous, neurologic, and growth abnormalities. Common additional clinical features are ichthyosis, intellectual/developmental disabilities, decreased fertility, ocular abnormalities, short stature, and recurrent infections. Within the spectrum of the TTD-related conditions appear a number of syndromes affecting mainly organs derived from the neuroectoderm. There are both photosensitive and nonphotosensitive forms of the disorder. TTD1 (ERCC2), TTD2 or Sabinas syndrome (ERCC3/XPB gene), TTD3 or Pollitt syndrome (GTF2H5 gene), TTD4 or BIDS syndrome (MPLKIP gene), TTD5 or IBIDS syndrome (RNF113A gene), SIBIDS syndrome, and ONMRS syndrome.

We report two unrelated patients with trichothiodystrophy type 4 from the same geographical area, carrying the same homozygous mutation in MPLKIP gene. First patient is a 7-year-old girl, first child of healthy nonconsanguineous parents (from the same town, 7000 habitants). Microcephaly (-4.74 SD), growth retardation, psychomotor delay, and brittle short growing hair. Optic microscopy showed pili torti and break stems. Homozygous c.277delT (p.Ser93Profs) mutation in MPLKIP gene was detected by exome sequencing. Patient 2 is a 2-year-old girl, second child of healthy unrelated parents, with psychomotor delay, microcephaly (-4.66 SD), limited growth and brittle hair. Hair analysis revealed trichoschisis and break stems. Due to highly concordant clinical features and geographical overlap with patient 1, analysis of Ser93Profs mutation on MPLKIP was performed first, detecting the same homozygous mutation, suggesting a founder effect in the area.

**P08.87****Mutations specific to the Rac-GEF domain of TRIO cause intellectual disability and microcephaly**R. Pengally<sup>1</sup>, S. Greville-Haygate<sup>1</sup>, E. Schmidt<sup>1</sup>, E. Seaby<sup>1</sup>, C. Fagotto-Kaufman<sup>1</sup>, R. Jabal-Ameli-Forooshani<sup>1</sup>, S. Mehta<sup>2</sup>, M. Parker<sup>3</sup>, D. Goudie<sup>4</sup>, C. Mercer<sup>1</sup>, The DDD Study, A. Debant<sup>1</sup>, S. Ennis<sup>1</sup>, D. Baralle<sup>1</sup>;<sup>1</sup>University of Southampton, Southampton, United Kingdom, <sup>2</sup>Addenbrookes Hospital, Cambridge, Cambridge, United Kingdom, <sup>3</sup>Sheffield Childrens Hospital, Sheffield, United Kingdom, <sup>4</sup>Tayside university Hospitals NHS trust, Dundee, United Kingdom.

**Background:** Neurodevelopmental disorders have challenged clinical genetics for decades, with over 700 genes implicated and many whose function remains unknown. The application of whole-exome sequencing is proving pivotal in closing the genotype/phenotype gap through the discovery of new genes and variants that help to unravel the pathogenic mechanisms driving neuropathogenesis. One such discovery includes TRIO, a gene recently implicated in neurodevelopmental delay. **Methods:** Whole-exome sequencing was undertaken on a family presenting with global developmental delay, microcephaly and mild dysmorphism. Father/daughter exome analysis was performed, followed by confirmatory Sanger sequencing and segregation analysis on four individuals. Three further patients were recruited through the deciphering developmental disorders (DDD) study. Functional studies were undertaken using patient-specific Trio protein mutations. **Results:** We identified a frameshift deletion in TRIO that segregated autosomally dominantly. By scrutinising data from DDD, we further identified three unrelated children with a similar phenotype who harboured de novo missense mutations in TRIO. Biochemical studies demonstrated that in 3 out of 4 families, the Trio mutations affected the Dbl homology domain and led to a markedly reduced Trio-mediated Rac1 activation. **Conclusion:** We describe an inherited global developmental delay phenotype associated with a frameshift deletion in TRIO. Additionally, we identify pathogenic de novo missense mutations in TRIO associated with the same overwhelming phenotype; intellectual disability, microcephaly, and dysmorphism with striking digital features. We further functionally validate the importance of the Dbl homology domain in Trio protein function. Our study demonstrates how genomic technologies are yet again proving prolific in diagnosing and advancing the understanding of neurodevelopmental disorders.

**P08.88****UNC80 mutation causes a syndrome of hypotonia, severe intellectual disability, dyskinesia and dysmorphism, similar to that caused by mutations in its interacting cation channel NALCN**

*Y. Perez<sup>1</sup>, R. Kadir<sup>1</sup>, M. Volodarsky<sup>1</sup>, I. Noyman<sup>2</sup>, H. Flusser<sup>3</sup>, Z. Shorer<sup>2</sup>, L. Gradstein<sup>4</sup>, R. Birnbaum<sup>5</sup>, O. Birk<sup>1,6</sup>;*

<sup>1</sup>The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel, <sup>2</sup>Pediatric Neurology unit, Division of Pediatrics, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel, <sup>3</sup>Zussman Child Development Center, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel,

<sup>4</sup>Department of Ophthalmology, Soroka Medical Center and Clalit Health Services, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel, <sup>5</sup>Department of Life Sciences and Faculty of Natural Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>6</sup>Genetics Institute, Soroka University Medical Center, Ben Gurion University of the Negev, Beer Sheva, Israel.

**Background:** A syndrome of profound hypotonia, intellectual disability, intrauterine growth retardation with subsequent failure to thrive, dyskinesia and epilepsy was diagnosed in Bedouin Israeli families. Mild dysmorphism was evident: plagiocephaly, broad forehead with prominent nose, smooth philtrum and congenital esotropia. We set out to decipher the molecular basis of this syndrome.

**Methods:** Genome wide linkage analysis and fine mapping were done. Whole exome sequencing data were filtered for candidate variants within locus. Validation and segregation of the mutation was assayed via Sanger sequencing. UNC80 expression pattern was analyzed through RT-PCR.

**Results:** Homozygosity mapping followed by fine mapping identified a 7.5 Mb disease-associated locus (LOD score 3.5) on chromosome 2. Whole exome and Sanger sequencing identified a single homozygous nonsense mutation within this locus, segregating within the families as expected for recessive heredity and not found in a homozygous state in 150 Bedouin controls: c.151C>T, p.(R51\*) in UNC80.

**Conclusions:** The syndrome described is caused by a mutation in UNC80, truncating most of the 3258 amino acids highly conserved encoded protein, that has no known motifs. UNC80 bridges between UNC79 and the cation channel NALCN, enabling NALCN's role in basal Na<sup>+</sup> leak conductance in neurons, essential for neuronal function. The phenotype caused by the UNC80 mutation resembles that previously described for homozygous NALCN mutations.

**P08.89****UNC80 mutations lead to a recognizable syndrome with persistent hypotonia, encephalopathy, severe intellectual disability and postnatal growth retardation**

*A. Stray-Pedersen<sup>1,2</sup>, J. Cobben<sup>3</sup>, T. E. Prescott<sup>4</sup>, S. Lee<sup>5</sup>, C. Cang<sup>5</sup>, K. Aranda<sup>6</sup>, S. Ahmed<sup>6</sup>, M. Alders<sup>7</sup>, T. Gerstner<sup>8</sup>, K. Aslaksen<sup>9</sup>, M. Tetreault<sup>10</sup>, W. Qin<sup>11</sup>, T. Hartley<sup>11</sup>, S. N. Jhangiani<sup>12</sup>, D. M. Muzny<sup>12</sup>, M. Tarailo-Graovac<sup>13</sup>, C. D. M. van Karnebeek<sup>14</sup>, J. R. Jupski<sup>15,12</sup>, D. Ren<sup>1</sup>, G. Yoon<sup>6</sup>;*

<sup>1</sup>Norwegian National Newborn Screening, Oslo University Hospital, Oslo, Norway,

<sup>2</sup>Baylor-Hopkins Center for Mendelian Genomics of the Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>3</sup>Department of Pediatrics, Academic Medical Center University Hospital, Amsterdam, Netherlands,

<sup>4</sup>Department of Medical Genetics, Oslo University Hospital, Oslo, Norway, <sup>5</sup>Department of Biology, University of Pennsylvania, Philadelphia, PA, United States, <sup>6</sup>Division of Clinical and Metabolic Genetics, The Hospital for Sick Children and University of Toronto, Toronto, ON, Canada, <sup>7</sup>Department of Clinical Genetics, AMC University Hospital, Amsterdam, Netherlands, <sup>8</sup>Department of Pediatrics, Sørlandet Hospital, Arendal, Norway, <sup>9</sup>Department of Pediatrics, Sørlandet Hospital, Kristiansand, Norway, <sup>10</sup>Department of Human Genetics, McGill University and Genome Québec Innovation Center, Montreal, QC, Canada, <sup>11</sup>The Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada, <sup>12</sup>Baylor College of Medicine Human Genome Sequencing Center, Houston, TX, United States, <sup>13</sup>Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, BC, Canada, <sup>14</sup>Department of Pediatrics and Centre for Molecular Medicine and Therapeutics, University of British Columbia, and Care4Rare Canada Consortium, Vancouver, BC, Canada, <sup>15</sup>Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, United States.

UNC80, a component of the NALCN sodium channel complex, interacts directly with NALCN and regulates the basal excitability of the nervous system. Loss-of-function mutations of NALCN cause autosomal recessive infantile hypotonia with psychomotor retardation and characteristic facies (IHPRF; MIM #615419). Animal models of UNC80 and NALCN deficiency have similar phenotypes to those reported in humans with biallelic mutations in NALCN. We report 4 affected individuals from 3 unrelated families of different ethnicity (Iraq, Morocco, Norway) with profound persistent hypotonia, severe intellectual disability, postnatal growth retardation +/- microcephaly and seizures due either to homozygous missense mutations (p.P177S,

p.R2536T) or compound heterozygous truncating mutations in UNC80. HEK293T cells transfected with the homozygous p.P1700S mutation showed markedly decreased NALCN currents compared to controls on patch clamp recording. Recently, two other groups reported in total 16 affected individuals from 6 families of Arab ethnicity (3 from Saudi Arabia, 1 from Egypt, and 2 related Negev Bedouin families) with homozygosity for either 1 missense (p.V189M) or 3 truncating mutations in UNC80, with phenotypes resembling our patients and patients with NALCN deficiency. Biallelic mutations in UNC80 result in an intellectual disability phenotype reminiscent of the severe end of the Angelman/Rett syndrome spectrum.

**P08.90****Whole exome sequencing of 28 individuals with likely autosomal recessive intellectual disability reveals truncating variants in previously reported ID-genes**

*R. Buchert<sup>1</sup>, R. Abou Jamra<sup>2</sup>, A. Baraghiti<sup>3</sup>, M. Sturm<sup>1</sup>, J. Sommerfeld<sup>1</sup>, C. Vogel<sup>1</sup>, C. Bauer<sup>1</sup>, B. Kootz<sup>1</sup>, S. Junker<sup>4</sup>, A. Jamal<sup>5</sup>, M. Hamdallah<sup>5</sup>, W. Al-Ameri<sup>6</sup>, A. Rieß<sup>3</sup>, P. Bauer<sup>1</sup>, O. Rieß<sup>3</sup>, T. Fröhlich<sup>3</sup>;*

<sup>1</sup>Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, <sup>2</sup>Institute of Human Genetics, University Medical Center Leipzig, Leipzig, Germany, <sup>3</sup>Department of Biotechnology and Genetic Engineering, Philadelphia University, Amman, Jordan, <sup>4</sup>private practice, Amman, Jordan, <sup>5</sup>Farah Hospital, Amman, Jordan, <sup>6</sup>Ibn Alhytham Hospital, Amman, Jordan.

In a bilateral project between Germany and Jordan, we examined 28 consanguineous Jordanian families with intellectual disability (ID) of probably autosomal recessive inheritance. We conducted whole exome sequencing (WES) for one affected individual per family and filtered for homozygous variants. With this approach we could identify homozygous truncating variants in the previously established ID-genes *NT5C2* (p.Gln447fs5), *ALDH5A1* (p.Gln362\*), *GPR56* (p.Trp657\*) and *WDR62* (p.Trp836\*).

Although family pedigrees suggested autosomal recessive ID, we were able to identify autosomal dominant causes in 2 families. In a consanguineous family with one affected individual, CNV analysis of WES coverage data revealed a de novo deletion on chromosome 4q21.22-21.23 which was previously reported in individuals with ID and fits the phenotype of the examined individual.

In another individual we identified a heterozygous truncating mutation in *TCF4* previously described in Pitt-Hopkins-syndrome. Segregation analysis revealed that the likewise affected sister also carries this variant while both parents are negative for this variant with no traces of somatic mosaicism. Thus, we propose that this variant is the result of a germline mosaic and causative for the phenotype.

In total, with our approach of WES of affected individuals with likely autosomal recessive ID, we identified the underlying causes of intellectual disability in 6 out of 28 families. However, in 2 families the pathogenic changes were due to de novo events. This stresses the importance of taking into account all possible patterns of inheritance including germline mosaics when analyzing WES data.

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**P08.91****A new mutation in KMT2A gene in a boy with Wiedemann-Steiner syndrome**

*M. Soeiro e Sá, P. Dias, A. B. Sousa;*

*Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisbon, Portugal.*

Wiedemann-Steiner syndrome (WSS) is a rare autosomal dominant intellectual disability (ID) syndrome characterized by hypotonia, growth deficiency, distinctive facial gestalt and hypertrichosis cubiti. It is due to heterozygous mutations in KMT2A (MLL) gene at 11q23.3, encoding a histone methyltransferase. Since the identification of the causative gene in 2012, only 20 cases have been reported, all caused by different mutations.

Herein we describe a 10-year-old boy with moderate ID, short stature (<5th centile), generalized obesity (BMI in the 95th centile), thick hair with frontal upswep, low posterior hairline, small and vertically narrow palpebral fissures, hypertelorism, bilateral epicanthus, wide nasal bridge, long and smooth philtrum, broad right first digit, bilateral sandal gap, cutaneous syndactyly between the second and third toes and bilateral long vellus hair on the elbows. Hearing and vision were unaffected. Karyotype, array-CGH and FMR1 molecular analysis were normal.

The phenotype suggested WSS and the diagnosis was confirmed with the identification of a heterozygous variant, c.502+1G>T, in KMT2A. The latter was previously unreported but considered likely pathogenic because it affects splicing.

Hairy elbows can be isolated or present in association with a spectrum of other features; when syndromic they are very suggestive of WSS and analysis of KMT2A is warranted. Etiological clarification classifies WSS within the large group of chromatin remodeling defect disorders, which are thought to result from global changes in protein expression during embryonic development leading to multiorgan abnormalities. How this mechanism can produce such a peculiar effect as localized hair growth on the elbows is still unclear.

#### P08.92

#### De novo Loss of Function Mutations in the KIAA2022 Gene on Xq13.3 Are Associated with Intellectual Disability, Impaired Language, and Epilepsy in Females

*A. Pujo<sup>1,2</sup>, R. Webster<sup>3</sup>, M. T. Cho<sup>2</sup>, K. Retterer<sup>2</sup>, F. Millan<sup>2</sup>, C. Nowak<sup>4</sup>, J. Douglas<sup>4</sup>, A. Amad<sup>5</sup>, G. Raymond<sup>6</sup>, M. Johnson<sup>6</sup>, A. Begtrup<sup>2</sup>, D. McKnight<sup>2</sup>, J. Juusola<sup>2</sup>, O. Devinsky<sup>7</sup>, W. Chung<sup>8</sup>,*

<sup>1</sup>IDIBELL, Barcelona, Spain, <sup>2</sup>GeneDx, Gaithersburg, MD, United States, <sup>3</sup>Department of Pediatrics, Columbia University Medical Center, New York, NY, United States, <sup>4</sup>Boston Children's Hospital, Boston, MA, United States, <sup>5</sup>University of Michigan, Ann Arbor, MI, United States, <sup>6</sup>Pediatric Neurology, University of Minnesota, Minnesota, MN, United States, <sup>7</sup>New York University School of Medicine, New York, NY, United States, <sup>8</sup>Department of Pediatrics, Columbia University Medical Center, New York, NY, United States.

Intellectual disability (ID) affects about 1%-3% of humans with a gender bias toward males. In the search for genes involved in monogenic ID, molecular characterization of chromosomal rearrangements involving the X-chromosome, and X-chromosome exome sequencing, have been important in the identification of the more than 100 genes to date. We report the results of diagnostic whole-exome sequencing in trios to identify five females carrying de novo predicted pathogenic mutations in KIAA2022 (OMIM: 300524), from 1500 females with ID/developmental delay. The encoded product, X-linked Intellectual Disability Protein Related to Neurite Extension (XPN), is highly expressed in the developing and adult brain and is involved in the regulation of neuronal migration and cell adhesion. Our five females come from unrelated families, and are characterized by severe intellectual disability, developmental delay, epilepsy refractory to treatment, impaired language, autism-spectrum disorder, hypotonia, and ataxic gait with normal brain MRIs. Additional but less common symptoms include gastrointestinal reflux, esotropia and mild dysmorphisms. The phenotypic severity in females is similar to that observed in the 13 previously reported males with pathogenic variants in KIAA2022, who were born from unaffected mother carriers. This report, together with three other recently described symptomatic female patients, contributes to the data supporting KIAA2022 as a cause of intellectual disability in females, and bears direct implications for genetic counseling and testing.

#### P08.93

#### A novel mutation in ZMYND11 gene in a girl with severe intellectual disability

*O. Moldovan<sup>1</sup>, C. Martins Halpern<sup>2</sup>, A. Sousa<sup>1</sup>,*

<sup>1</sup>Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Lisbon, Portugal, <sup>2</sup>Unidade de Neuropediatria, Centro de Desenvolvimento, Hospital Garcia de Orta, Lisbon, Portugal.

Introduction: Mental retardation, autosomal dominant 30 (MRD30) (OMIM#616083) is caused by mutations in the ZMYND11 gene. Less than ten cases have been previously reported with mutations in this gene. We describe a girl with severe global developmental delay and dysmorphism. Materials and Methods: She was born to healthy and nonconsanguineous parents at 39 weeks gestation. Family history was unremarkable. Her birth weight was 2740g (10th centile), length 47,5cm (25th centile) and head circumference 32cm (3rd centile). Ventricular disproportion and esophageal atresia were suspected in the prenatal scans, but not confirmed after birth. On the other hand, she presented facial dysmorphisms, VSD and axial hypotonia. She also had gastro-esophageal reflux, which motivated recurrent episodes of hospitalization for apparent life-threatening events (ALTE), and drug-resistant sleep disturbance. At 1 year and 3 months, her weight and length were above the 75th centile and head circumference was on the 50th centile. She had severe developmental delay with incomplete head control and inability to maintain a sitting position. We performed arrayCGH which revealed no significant CNV, and FISH analysis in buccal mucosa cells and skin fibroblasts to exclude tetrasomy 12p. Finally we decided for trio exome sequencing and a de novo likely-causative variant was detected (c.1259C>A; p.Ser420Tyr) in ZMYND11 gene, predicted to be pathogenic and previously unreported in population databases.

Conclusions: We report a patient presenting a new mutation in ZMYND11 gene which determines the most severe phenotype described to date.

#### P09 Neurogenetic and psychiatric disorders

##### P09.001

##### Characterization of rearrangements in the 22q11.2 region: A population-based screening of 25,703 newborn Danes.

*T. Sparsø<sup>1</sup>, L. Olsen<sup>1</sup>, S. Weinsheimer<sup>1</sup>, M. Bertalan<sup>1</sup>, W. Mazin<sup>1</sup>, J. Grauholm<sup>2</sup>, M. Hollegaard<sup>2</sup>, D. Hougaard<sup>2</sup>, C. B. Pedersen<sup>3</sup>, P. B. Mortensen<sup>3</sup>, W. Thompson<sup>1</sup>, BROAD investigators, iPSYCH investigators, T. Werge<sup>1</sup>,*

<sup>1</sup>Biological Psychiatry, Sct Hans, Roskilde, Denmark, <sup>2</sup>Section of Neonatal Genetics and the Danish Neonatal Screening Biobank, Copenhagen, Denmark, <sup>3</sup>National Centre for Register-based Research, Aarhus, Denmark.

Purpose: Genetic rearrangements in the 22q11.2 region are mediated by LCR. Frequency estimates of the 3Mb deletion has so far been based on hospital recruitments and commonly referred to as 1:4000 newborns. A unique source for population-based studies is provided by the iPSYCH initiative ([www.ipsych.au.dk](http://www.ipsych.au.dk)). Dried bloodspots from all newborns have been stored since 1982 and today DNA has been extracted from 76,109 individuals (N=25,703 are random samples). Here, we present the first accurate population-based prevalence of both 22q11.2 deletions and duplications. Using the National Health Registry, we provide population-based incidence rate ratio (IRR) estimates for neurodevelopmental disorders in individuals with the 22q11.2 rearrangement. We used incidence density sampling to calculate incidence rate ratio (IRR) estimates for the psychiatric diagnostic categories (ADHD, autism, schizophrenia, and mental retardation). Results: Among the 25,703 population-based samples we observed a frequency of 0.027% (1:3672) for 22q11.2 deletions and 0.0622% (1:1606) for the reciprocal duplications. In our samples of ADHD (N=16,715), autism spectrum (N=14,333), schizophrenia (N=2,623) and mental retardation (N=4,097), we found a significant increased IRR for individuals carrying the 22q11.2 deletion (ADHD: IRR=5.00 (p=0.0026), autism: IRR=6.00 (p=0.00309), mental retardation: IRR=14.29 (p<0.00001). Carriers of the reciprocal 22q11.2 duplication showed similar increased risk for neurodevelopmental disorders (ADHD: IRR=3.75 (p=0.00053), autism: IRR=2.86 (p=0.018), schizophrenia: IRR=4.29 (p=0.035), and mental retardation: IRR=4.10 (p=0.00398), however, not as profound as for deletion carriers. Conclusion: True population-based frequency of the 22q11.2 deletion is marginally higher than the widely accepted 1:4000. Both the 22q11.2 deletion/duplication confer risk of neurodevelopmental disorders at the population-level.

##### P09.002

##### 22q11.2 deletion syndrome and HTRA2 p.G399S in a patient with juvenile onset Parkinson disease

*P. Prontera<sup>1</sup>, V. Napolioni<sup>2</sup>, D. Roggia<sup>1</sup>, I. Isidori<sup>1</sup>, A. Mencarelli<sup>1</sup>, N. Tambasco<sup>3</sup>, G. Merla<sup>4</sup>, G. Stangoni<sup>1</sup>, P. Calabresi<sup>1,5</sup>,*

<sup>1</sup>Medical Genetics Unit, Perugia, Italy, <sup>2</sup>Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, CA, United States,

<sup>3</sup>Neurologic Clinic, University of Perugia, Perugia, Italy, <sup>4</sup>Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy, <sup>5</sup>IRCCS Fondazione „S. Lucia”, Rome, Italy.

In a patient with 22q11.2 deletion syndrome who developed at 32 years of age Parkinson Disease (PD), with tremor as the predominant symptom, we performed a WES, on Illumina Hiseq1500 after prepping the samples with a Nextera Rapid Capture Exome kit (Illumina) and an array-CGH analysis (CytoChip 4x180K, BlueGnome) of the trio. The array-CGH better defined the extent of the *de novo* deletion (from 18,894,865 to 21,505,388 [hg19]), while WES did not disclose mutations in known PD genes, but identified the controversial HTRA2 p.G399S variant in the heterozygous state, inherited from the healthy mother. HTRA2 is a serine protease located in the inter-membrane space of the mitochondria and previous studies demonstrated that HTRA2 p.G399S leads to mitochondrial dysfunction, altered mitochondrial morphology and decreased protease activity. The region 22q11.2 harbors six genes encoding mitochondrial proteins, namely, PRODH, MRPL40, SLC25A1, TANGO2, TXNRD2, and ZDHHC8, that we showed to be down regulated in our patient, by a whole transcriptome analysis on RNA extracted from a fibroblast culture obtained from skin biopsies of the patient and of an age and sex-matched control (Unrestricted Gene expression Human Microarray 4x44K, Agilent Technologies). Even if could be conceivable that a severe mitochondrial dysfunction caused by the concomitant defects in these genes may be the cause of the juvenile PD in our patient, nevertheless this single case report cannot allow for firm conclusions, but it provides additional starting points for future cross-correlations and investigations of genetic epistasis, especially between rare genetic cause of PD.

## P09.003

### 3p- syndrome with deletion of CHL1, CNTN6 and first exon of CNTN4 gene: phenotype and genotype relationship in a 6 yo boy

A. Lafuente, M. Ortiz, J. Juan, G. Pi, A. Zúñiga;  
Hospital de la Ribera, Alzira (Valencia), Spain.

The 3p deletion syndrome is a rare contiguous gene syndrome caused by deletions in the 3p25-pter region. The deletions are variable in size, they don't present common break points and mostly occur de novo, but a few familial cases have been reported. The syndrome is characterized by a recognizable phenotype including low birth weight, growth and mental retardation, developmental delay and characteristic facial appearances. The clinical manifestations in individuals with 3p deletions vary from normal to severe. A milder phenotypic effect or a normal intelligence has also been described for larger, often inherited, deletions of this region and appears to be secondary to the breakpoint's location and the deletion extent. Proband is a 6 yo male and he is the second child of healthy, non-consanguineous parents. Karyotype was normal male. No family history of congenital anomalies or mental retardation was referred. The child was born after 36 weeks of uneventful pregnancy, by caesarean section. He showed a regular physical and psychomotor development (sitting at 6 months, walking at 14 months). At school learning difficulties were observed and a neuropsychological evaluation showed a borderline I.Q. level and language disorders. CGH microarray analysis showed a sub microscopic 3p26.3 terminal deletion. Parents were normal. The imbalance is less than 2 Mb in size and includes three OMIM gene: CHL1 (607416), CNTN6 (607220) and first exon of CNTN4 (607280). CNTN4 plays an important role in the neuronal network development. CHL1 has been suggested to be responsible for mental defects in patients with 3p- syndrome.

## P09.004

### Association of AADAC deletion and Gilles de la Tourette syndrome in a large European cohort

B. Bertelsen<sup>1</sup>, H. Stefansson<sup>2</sup>, L. R. Jensen<sup>3</sup>, L. Melchior<sup>4</sup>, N. M. Debes<sup>4</sup>, L. Skov<sup>4</sup>, C. Groth<sup>4</sup>, T. Werge<sup>5</sup>, I. Karagiannidis<sup>6</sup>, Z. Tarnok<sup>7</sup>, C. Barta<sup>8</sup>, P. Nagy<sup>9</sup>, L. Farkas<sup>9</sup>, K. Brondum-Nielsen<sup>10</sup>, R. Rizzo<sup>9</sup>, M. Gulisano<sup>9</sup>, D. Rujescu<sup>10</sup>, L. A. Kiemeney<sup>11</sup>, S. Tosato<sup>12</sup>, M. N. Nawaz<sup>2</sup>, A. Ingason<sup>2</sup>, U. Unnsteinsson<sup>13</sup>, S. Steinberg<sup>2</sup>, P. Ludvigsson<sup>2</sup>, K. Stefansson<sup>2</sup>, A. W. Kuss<sup>3</sup>, P. Paschou<sup>6</sup>, D. Cath<sup>13</sup>, P. J. Hoekstra<sup>14</sup>, K. Müller-Vahl<sup>15</sup>, M. Stuhrman<sup>16</sup>, A. Silahtaroglu<sup>17</sup>, R. Pfundt<sup>18</sup>, Z. Tümer<sup>1</sup>;

<sup>1</sup>Kennedy, Center, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, <sup>2</sup>deCode Genetics, Reykjavik, Iceland,

<sup>3</sup>Department of Human Genetics, University Medicine Greifswald and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany, <sup>4</sup>Tourette Clinic, Department of Pediatrics, Herlev Hospital, Herlev, Denmark,

<sup>5</sup>Institute of Biological Psychiatry, Mental Health Centre, Sct. Hans, MHS – Capital Region of Denmark; Institute of Clinical Sciences, Faculty of Medicine and Health Sciences, University of Copenhagen; iPSYCH – The Lundbeck Foundation's Initiative for Inte, Copenhagen, Denmark, <sup>6</sup>Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupoli, Greece, <sup>7</sup>Vadaskert Child and Adolescent Psychiatry Clinic, Budapest, Hungary, <sup>8</sup>Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary,

<sup>9</sup>Section of Child Neuropsychiatry, Department of Medical and Pediatric Sciences, Catania University, Catania, Italy, <sup>10</sup>Department of Psychiatry, Psychotherapy and Psychosomatics, Martin-Luther-University, Halle, Germany, <sup>11</sup>Department for Health Evidence, Radboud University Medical Center, Nijmegen, Netherlands, <sup>12</sup>Section of Psychiatry, Department of Public Health and Community Medicine, University of Verona, Verona, Italy, <sup>13</sup>Department of Clinical and Health Psychology, Utrecht University & Altrecht Academic Anxiety Outpatient Clinics, Utrecht, Netherlands, <sup>14</sup>University of Groningen, University Medical Center Groningen, Department of Psychiatry, Groningen, Netherlands, <sup>15</sup>Clinic of Psychiatry, Socialpsychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany, <sup>16</sup>Institute of Human Genetics, Hannover Medical School, Hannover, Germany, <sup>17</sup>Wilhelm Johansen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine,

**Background:** Gilles de la Tourette syndrome (GTS) is a complex neuropsychiatric disorder with a strong genetic influence where copy number variations (CNVs) are suggested to play a role in disease pathogenesis. In a previous small scale CNV study of a GTS cohort (n=111), recurrent exon-affecting microdeletions of four genes, including the gene encoding arylacetamide deacetylase (AADAC), were observed and merited further investigations. **Methods:** We screened a Danish cohort of 243 GTS patients and 1,571 controls for submicroscopic deletions and duplications of these four genes. The most promising candidate gene, AADAC, identified in this Danish discovery sample was further investigated in cohorts from Iceland, the Netherlands, Hungary, Germany and Italy, and a final meta-analysis including a total of 1,181 GTS patients and 118,730 controls from these six European countries was performed. Subsequently, expression of the candidate gene in the central nervous system was investigated using human and mouse brain tissues. **Results:** In the Danish cohort, we identified eight patients with overlapping deletions of AADAC. Investigation of the additional five countries showed

a significant association between the AADAC deletion and GTS, and a final meta-analysis confirmed the significant association ( $P=4.4 \times 10^{-4}$ ;  $OR=1.9$ ; 95% CI 1.33-2.71). Furthermore, RNA in situ hybridization and RT-PCR studies revealed that AADAC is expressed in several brain regions previously implicated in GTS pathology.

**Conclusions:** AADAC is a candidate susceptibility factor for GTS and the present findings warrant further genomic and functional studies to investigate the role of this gene in the pathogenesis of GTS.

## P09.005

### Reduced penetrance of p.Thr585Met RANBP2 mutation in autosomal dominant acute necrotizing encephalopathy

D. R. Carvalho, B. J. A. F. Martins, C. E. Speck-Martins, J. M. Brum, A. La Rocque-Ferreira; SARAH Network of Rehabilitation Hospitals, Brasilia, Brazil.

Acute necrotizing encephalopathy (ANE) is clinically characterized by fever, acute encephalopathy, seizures, and rapid progression to coma within days of onset of a viral illness in otherwise healthy children, with no evidence of central nervous system infection. Brain magnetic resonance imaging (MRI) shows multiple symmetrical lesions affecting primarily the thalamus but also brainstem, putamina, periventricular white matter and cerebellum. Most ANE is sporadic and nonrecurrent. A missense mutation in *RANBP2* gene has been identified as a major cause of familial and recurrent ANE (MIM 608133), also named autosomal dominant ANE (ADANE). Only few families with ADANE have been reported.

Clinical and radiological findings of six members of a two-generation ADANE family were described. Sequencing revealed the c.1754C>T *RANBP2* mutation (p.Thr585Met) in five of them.

DNA was not obtained from radiological affected boy who died at age 13 months after the second episode of coma. His 18-year-old brother manifests spastic quadripareisis, dystonia and moderate mental retardation as sequelae. Their healthy mother (44 years) is the sister of a 42-year-old heterozygous father of three affected girls and he also never had any symptom. After acute encephalopathy episodes, the 11-year-old sister presents mild dysmetria and cognitive impairment with strabismus, while the 10-year-old sister has spastic diparesis, ataxia and mental retardation. The 7-year-old heterozygous sister manifested unexplained decreased level of consciousness during two viral febrile infections. She had a complete recovery and normal brain MRI.

We reinforce the incomplete penetrance of ADANE and great variability regarding neurologic damage and residual signs.

## P09.006

### Identification of ADHD candidate genes in large pedigrees combining linkage analysis and whole-exome sequencing

J. Corominas Galbany<sup>1</sup>, M. Klein<sup>1</sup>, T. Zayats<sup>2</sup>, S. Gross-Lesch<sup>3</sup>, M. Pauper<sup>4</sup>, C. Jacob<sup>3</sup>, A. Reif<sup>4</sup>, M. Romanos<sup>5</sup>, A. Arias Vasquez<sup>1,6</sup>, K. Lesch<sup>3</sup>, B. Franke<sup>1,7</sup>;

<sup>1</sup>Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Department of Biomedicine, Bergen University, Bergen, Norway,

<sup>3</sup>Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany, <sup>4</sup>Department of Psychiatry, Psychosomatics and Psychotherapy, University of Frankfurt am Main, Frankfurt, Germany, <sup>5</sup>Department of Child Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany,

<sup>6</sup>Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, Netherlands, <sup>7</sup>Department of Psychiatry, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, Netherlands.

Attention-Deficit/Hyperactivity Disorder (ADHD) is a common neuropsychiatric and multifactorial disorder characterized by inattention, and/or motor hyperactivity and impulsivity. The study of multi-generation pedigrees with multiple affected individuals can point towards novel ADHD-related genes. By combining haplotype analysis and whole exome sequencing (WES), we aim to identify genes carrying rare and/or common variants that contribute to ADHD. Three German pedigrees with multiple ADHD-affected adults were used in this study, in which linkage analysis was carried out on the major part of the individuals and WES data from two or more patients were obtained to detect shared genetic variants. Genotyping data was used to identify haplotype blocks segregating with disease. Linkage analyses were performed using a Multi-Point analysis implemented in Superlink online SNP, for each family and by combining them. Regions with a maximum LOD score higher than 2 were selected as a candidate regions. Genes in these regions containing shared rare genetic variants were selected as candidate genes. A common problem in multifactorial diseases is that the effect of individual markers is too weak to be detected; consequently, multiple genetic markers may be simultaneously analyzed to increase explained

variance. Therefore, gene-set analyses were performed in an independent exome-chip dataset from 1846 ADHD patients and 7519 controls (IMpACT). The gene-set from one family indeed showed a significant association with adult ADHD, suggesting that the variants in several genes might act together in increasing ADHD risk. The analysis strategy followed here might provide new knowledge for a better understanding of ADHD's etiology.

#### P09.007

##### Shared genetic effects between clinical ADHD and smoking, alcohol and breastfeeding in mothers from the general population

*E. Stergiakouli<sup>1,2</sup>, J. Martin<sup>3</sup>, M. Hamshere<sup>3</sup>, B. St Pourcain<sup>1,4</sup>, N. Timpson<sup>1</sup>, A. Thapar<sup>3</sup>, G. Davey Smith<sup>1</sup>;*

<sup>1</sup>MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, United Kingdom,

<sup>2</sup>School of Oral and Dental Sciences, University of Bristol, Bristol, United Kingdom,

<sup>3</sup>Institute of Psychological Medicine and Clinical Neurosciences, MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Cardiff, United Kingdom, <sup>4</sup>Max Planck Institute for Psycholinguistics, Nijmegen, Netherlands.

**Introduction:** Prenatal smoking and alcohol consumption have been suggested as possible risk factors for ADHD. Inferring causality has not been possible because the mother provides both the prenatal environment and genetic risk factors for ADHD. We investigated if there are shared genetic effects between ADHD and possible risk factors for ADHD using polygenic risk score analysis in the general population.

**Materials and Methods:** ADHD polygenic risk scores were calculated for 8340 mothers from the Avon Longitudinal Study of Parents and Children (ALSPAC) based on the results of a genome-wide ADHD case-control study (Stergiakouli et al. 2012) and tested for association with smoking and alcohol consumption before and during pregnancy and with breastfeeding status.

**Results:** Higher genetic risk for ADHD was associated with higher odds of smoking before and during pregnancy and not breastfeeding (see Table). There was no evidence of association with alcohol consumption. Adjusting for ADHD polygenic score of the child did not change the association.

**Conclusions:** Our results show for the first time that there are shared genetic effects between ADHD and life style choices in mothers from the general population without the disorder. The mother can not only transmit genetic risk for ADHD to her offspring but also expose the child to risk factors through her life style choices that are in turn influenced by her genetic risk for ADHD.

Outcome	N	OR (95% CIs)	p value
Smoking before pregnancy	7530	1.05 (1.01 to 1.1)	0.03
Smoking at first trimester	7543	1.08 (1.03 to 1.15)	0.002
Alcohol consumption before pregnancy	7530	0.99 (0.91 to 1.08)	0.9
Alcohol consumption at first trimester	7543	0.98 (0.94 to 1.03)	0.5
Breastfeeding	6604	1.06 (1.01 to 1.11)	0.03

#### P09.008

##### Sex-specific genome-wide common variant analyses of attention deficit hyperactivity disorder

*J. Martin<sup>1,2,3</sup>, R. Walters<sup>2</sup>, D. Demontis<sup>4</sup>, M. Mattheisen<sup>4</sup>, S. Lee<sup>5,6</sup>, M. C. O'Donovan<sup>3</sup>, E. Robinson<sup>2</sup>, Psychiatric Genomics Consortium: ADHD Subgroup, iPSYCH-SSI-Broad ADHD Workgroup, B. Franke<sup>7</sup>, A. Borglum<sup>4</sup>, S. V. Faraone<sup>8</sup>, A. Thapar<sup>3</sup>, B. M. Neale<sup>2</sup>;*

<sup>1</sup>Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>Broad Institute, Cambridge, MA, United States, <sup>3</sup>Cardiff University, Cardiff, United Kingdom, <sup>4</sup>Aarhus University, Aarhus, Denmark, <sup>5</sup>University of New England, Armidale, Australia, <sup>6</sup>Queensland Brain Institute, Brisbane, Australia, <sup>7</sup>Radboud University Medical Center, Nijmegen, Netherlands, <sup>8</sup>SUNY Upstate Medical University, Syracuse, NY, United States.

Attention deficit hyperactivity disorder (ADHD) shows substantial heritability as measured by twin/family studies and genome-wide association studies (GWAS). ADHD is about four times more common in males than females. What is unclear is the nature of the apparent female protective effect. One hypothesis is that female cases carry a higher burden of genetic risk. Alternatively, the sources of variation are different between the sexes. We examined these two hypotheses using genome-wide data from the Psychiatric Genomics Consortium and the iPSYCH Project in collaboration with the Danish Neonatal Screening Biobank.

Initial sex-specific GWAS meta-analysis of ~11,000 ADHD cases (24% female) and ~21,000 population controls/pseudo-controls (51% female) showed no genome-wide significant variants for either sex. Genetic correlation analyses of female and male ADHD using LD-score regression and GREML showed a strong bivariate heritability:  $R_g$  (SE) ~1.0(0.2). Results examining the relative burden of common genetic risk variants, using a leave-one-sample-out polygenic risk score analysis approach, showed higher mean polygenic scores in cases than in controls but no association of ADHD genetic risk with sex in cases.

The high genetic correlation suggests that the vast majority of common variant risk is shared between females and males in ADHD and supports combining GWAS data from both sexes in meta-analyses. Further, these results suggest that common genetic variants do not appear to play a significant role in the sexual dimorphism of ADHD. Although no significant difference in polygenic risk has been detected, these analyses are still severely limited in power and will benefit from increasing sample sizes. (Wellcome-Trust:106047)

#### P09.009

##### The effect of parental age on the presence of de novo mutations - Lessons from neurofibromatosis type 1

*S. Ben-Shachar<sup>1</sup>, T. Dubov<sup>2</sup>, H. Toledano-Alhadeff<sup>3</sup>, F. Bokstein<sup>3</sup>, S. Constantini<sup>1</sup>;*

<sup>1</sup>The Genetic Institute, Tel-Aviv, Israel, <sup>2</sup>Sackler Faculty of Medicine, Tel-Aviv, Israel, <sup>3</sup>Tel-Aviv Medical Center, Tel-Aviv, Israel, <sup>4</sup>Tel-Aviv Medical Center, Tel-Aviv, Israel.

**Background:** Neurofibromatosis type 1 (NF1) is the most common autosomal dominant neurocutaneous disease with a prevalence of 1:2,500. Approximately 50% of cases are sporadic. Advanced paternal age is associated with germline mutations and autosomal diseases. We aimed to use NF1 as a paradigm to study the effect of parental age on sporadic mutations rates for both advanced and younger parental ages.

**Methods:** The medical charts of 118 NF1 pediatric patients followed in a specialized Israeli NF1 clinic were evaluated. Thirty-one cases were diagnosed by genetic tests and 87 by NIH clinical criteria. Sixty-four cases (54%) had a negative family history of NF1 (sporadic cases). Data on parental ages at the time of the children's birth were compared to the national population database.

**Results:** Significantly fewer fathers had been 25-29 years old at their child's birth compared with fathers in the general population (7.8% versus 21%, respectively,  $p=0.009$ ), and significantly more were  $\geq 40$  years old (29.7% versus 13.6%, respectively,  $p=0.0002$ ). Differences in maternal age between these two groups were less prominent.

**Conclusion:** The risk for sporadic NF1 was lower when the fathers were younger at the time of the affected child's birth and gradually increased with paternal age.

#### P09.010

##### Differential expression of inflammatory cytokines in peripheral blood mononuclear cells in early and late onset Alzheimer's disease

*N. Unaltuna<sup>1</sup>, G. Guven<sup>1</sup>, E. Lohmann<sup>2</sup>, H. Hanagasi<sup>2</sup>, E. Ozer<sup>1</sup>;*

<sup>1</sup>Department of Genetics, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey, <sup>2</sup>Behavioural Neurology and Movement Disorders Unit, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

**Introduction:** Neuroinflammation is involved in the Alzheimer's disease (AD) pathology. Our major focus was to clarify whether peripheral inflammation plays important roles in AD pathogenesis, particularly if there is a difference in expression of inflammatory cytokines in early vs late onset Alzheimer's patients (EOAD vs LOAD). For this, we analyzed the expression pattern of IL-1 $\beta$ , IL-6, IL-10, IL-12, TGF- $\beta$  and TNF- $\alpha$  in peripheral blood mononuclear cells of EOAD, LOAD and controls.

**Materials-Methods:** The study group consisted of 13 EOAD, 14 LOAD and 13 control patients. Quantitative Real Time PCR of each of the mRNA normalized with GAPDH was used to determine levels of cytokines. Parametric and non-parametric statistical tests were used for comparison of groups and correlations were analyzed using Pearson's linear correlation test.

**Results:** IL-1 $\beta$ , IL-6 and TNF- $\alpha$  were found to be significantly down-regulated in EOAD when compared with the LOAD and the controls. Decrease of IL-12 expression was observed in EOAD when compared with LOAD. The correlation analyses revealed that IL-6 was inversely correlated with TGF- $\beta$  for EOAD, for LOAD, however, IL-6 with IL-10 and IL-12 with TGF- $\beta$  were positively correlated. Such differences are present in PSEN1 positive and negative patients as well.

**Conclusions:** This is the first study comparing expression levels of peripheral cytokines between EOAD and LOAD patients. Our results suggest that EOAD and LOAD have differences in immune response, which might be monitored in peripheral blood cells as biomarkers. Further studies with larger population are needed to explore the underlying mechanisms of our findings.

## P09.011

### Expression of inflammation-related miRNAs and their selected target genes in peripheral blood mononuclear cells of early and late onset Alzheimer disease patients

**G. Guven<sup>1</sup>, E. Lohmann<sup>2</sup>, H. Hanagasi<sup>2</sup>, C. Gulec<sup>3</sup>, E. Ozer<sup>4</sup>, N. Unaltuna<sup>1</sup>;**

<sup>1</sup>Department of Genetics, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey, <sup>2</sup>Behavioural Neurology and Movement Disorders Unit, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey,

<sup>3</sup>Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

**Introduction:** Alzheimer Disease (AD) brains display a changed miRNA-expression-profile which is reflected also in peripheral blood mononuclear cells (PBMC). Therefore, we investigated the expression levels of inflammation-related miRNAs and their selected target genes in PBMC of early-onset AD (EOAD), late-onset AD (LOAD) patients and controls.

**Materials-Methods:** The study group consisted of 13 EOAD patients including 7 with [PSEN1(+)] and 6 without [PSEN1(-)] PSEN1 mutations, 14 patients with LOAD and 13 healthy, non-correlated controls. qRT-PCR determined levels of mir-146, mir-144, mir-34a and their target genes: TRAF6, TSPAN12, ADAM10, BECLIN1, TREM2. Statistical analyses were performed using SPSS software.

**Results:** mir-144 was significantly overexpressed whereas its target genes, ADAM10 and BECLIN1, were significantly down-regulated in PSEN1(-) EOAD group compared with the others.

In contrast, mir-34a was significantly down-regulated in PSEN1(+)-EOAD compared with LOAD and its target gene TREM2 levels were found to be significantly decreased in PSEN1(+)-EOAD when compared with controls, but not with LOAD.

mir-146a was significantly down-regulated in PSEN1(+)-EOAD compared with LOAD. The levels of its target genes, TSPAN12 and TRAF6, were found to be significantly down-regulated in PSEN1(-)-EOAD group compared with LOAD. Additionally, mir-146a expression was inversely correlated with TRAF6 in the LOAD group.

**Conclusions:** This is the first study comparing miRNAs and their target gene expression in PBMC of EOAD and LOAD patients. Our results suggest a different role of miRNAs in EOAD compared to LOAD. In addition, the presence/absence of PSEN1 mutation might also influence their regulation. However, studies including larger patient groups are requested to confirm our findings.

## P09.012

### Screening for preclinical Alzheimer's disease

**D. Farouk<sup>1</sup>, M. Shehab<sup>1</sup>, M. Mekkawy<sup>1</sup>, H. Afifi<sup>1</sup>, M. Ragab<sup>2</sup>, H. Awadallah<sup>2</sup>, M. Afifi<sup>3</sup>, S. Temtamy<sup>1</sup>;**

<sup>1</sup>The National Research Center, Cairo, Egypt, <sup>2</sup>Department of Environmental Medical Science, Institute of Environmental Research and Studies, Ain Shams University, Cairo, Egypt, <sup>3</sup>Institute of Psychiatry, Ain Shams University, Cairo, Egypt.

**Introduction:** The majority of Alzheimer's disease (AD) cases arise through interactions among genetic and environmental factors, the disease usually begins many years before clinical symptoms appear. Premature centromere separation (PCS) and micronuclei (MNi) are useful cytogenetic biomarkers for prediction and early diagnosis. Constricted life space and fewer years of education are two environmental risk factors for AD, avoiding the environmental factors in addition to early detection can significantly decrease the incidence of the disease.

**Materials and Methods:** This study was conducted on 26 patients diagnosed as AD and a group of 26 matched controls. All subjects performed life space measurement, Mini-Mental State Examination (MMSE), conventional cytogenetic analysis, Fluorescent In Situ Hybridization (FISH) analysis and Cytokinesis-block micronucleus assay (CBMN).

**Results:** The average years of education were significantly fewer in the patients group ( $P = 0.0466$ ) and their average life space was constricted compared to controls ( $P = 0.0234$ ). Significant positive correlation was found between score of MMSE and score of life space trajectories among the patients ( $r = 0.7886$ ) which demonstrates the effect of constricted life space on cognitive impairment. Cytogenetic results revealed a significant difference in percentage of PCS and score of MNi between AD patients and controls ( $P < 0.0001$ ).

**Conclusions:** These results clarify that screening for AD by the environmental risk factors and the cytogenetic biomarkers can be valuable for early detection as chromosomal instability occurs many years before evidence of clinical symptoms, and treatment is beneficial at this stage.

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## P09.013

### Liver X Receptor genes variants modulate risk and phenotype of ALS

**K. Mouzat<sup>1,2</sup>, N. Molinari<sup>3</sup>, J. Kantar<sup>4</sup>, A. Polge<sup>1</sup>, P. Corcia<sup>4</sup>, P. Couratier<sup>5</sup>, P. Clavelou<sup>6</sup>, R. Juntas-Morales<sup>2,7</sup>, N. Pageot<sup>2,7</sup>, J. A. Lobaccaro<sup>8</sup>, C. Raouf<sup>7</sup>, S. Lumbruso<sup>1,2</sup>, W. Camu<sup>2,7</sup>;**

<sup>1</sup>Laboratoire de Biochimie et Biologie Moléculaire, CHU Nîmes, Nîmes Cedex 9, France,

<sup>2</sup>INSERM UMR1051, Institut des Neurosciences de Montpellier, Hôpital Saint Eloi, Montpellier, France, <sup>3</sup>IMAG, UMR 5149, DIM, CHRU de Montpellier, Montpellier, France,

<sup>4</sup>Centre SLA, Service de Neurologie et Neurophysiologie Clinique, CHRU de Tours, UMR INSERM U930, Tours, France, <sup>5</sup>Centre SLA, Service de Neurologie, CHU Limoges, Limoges, France, <sup>6</sup>Centre SLA, Service de Neurologie A, CHU Clermont-Ferrand, Clermont-Ferrand, France, <sup>7</sup>ALS Center, Département de Neurologie, CHU Gui de Chauliac, Montpellier, France, <sup>8</sup>Génétique Reproduction et Développement, Clermont Université, Clermont-Ferrand, France; CNRS, UMR 6293, GReD, Aubière, France; INSERM, UMR 1103, GReD, Aubière, France; Centre de Recherche en Nutrition Humaine d'Auvergne, Clermont-Ferrand, France.

**Background:** Amyotrophic lateral sclerosis (ALS) is one of the most severe neurodegenerative disorder, characterized by the loss of motor neurons. Phenotype of ALS patients is highly variable, and suffers from the lack of reliable molecular markers. Lipid metabolism has recently been suggested as a potent modulating factor of ALS progression. The two isoforms of nuclear receptors Liver X Receptors (LXRs), LXR alpha (LXR $\alpha$ ) and LXR beta (LXR $\beta$ ) play a central role in lipid metabolism as cholesterol sensors. Several lines of evidence have also pointed LXRs as potential candidates to explain ALS phenotype variability. Subsequently, an association study using single nucleotide polymorphisms (SNPs) was conducted to explore LXRs as genetic makers for ALS.

**Results:** Associations between SNPs of LXR $\alpha$  (rs2279238 and rs7120118) and LXR $\beta$  (rs35463555 and rs2695121) genes and ALS were assessed by genotyping using high-resolution melting analysis in a cohort of 438 ALS patients and 330 healthy controls. The two LXR $\alpha$  SNPs rs2279238 and rs7120118 were shown to be associated with ALS risk. Analysis of clinical parameters revealed that the two LXR $\alpha$  SNPs were associated with age at onset in ALS patients ( $p = 0.0027$  and  $p = 0.0298$  respectively). This association was also strengthened by the number of rare alleles carried by patients. Moreover, rs2695121 within LXR $\beta$  gene was shown to be associated with disease duration ( $p = 0.0055$ ).

**Conclusions:** These findings reveal a new genetic association between LXRs and both ALS risk and phenotype, opening new perspectives in the understanding of molecular mechanisms underlying the disease.

## P09.014

### Association of FTO gene variants with sporadic amyotrophic lateral sclerosis in Greek patients

**K. Mitropoulos<sup>1</sup>, C. Mizzit<sup>2</sup>, E. Tsermpini<sup>3</sup>, E. Merkouri-Papadima<sup>3</sup>, A. Balasopoulou<sup>3</sup>, V. Dellatola<sup>3</sup>, V. Karathanasi<sup>3</sup>, T. Katsila<sup>3</sup>, G. P. Patrinos<sup>3</sup>;**

<sup>1</sup>University of Athens School of Medicine, Athens, Greece, <sup>2</sup>University of Malta, Msida, Malta, <sup>3</sup>University of Patras Faculty Health Sciences Department of Pharmacy, Patras, Greece.

Amyotrophic Lateral Sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons in the motor cortex, brain stem and spinal cord. Patients develop progressive paralysis resulting in death, usually caused by respiratory failure. Until now, no effective therapeutic strategy exists. It is well documented that sporadic ALS is a multifactorial disease, in which a genetic component, such as SOD1, FTO, FUS, TARDP, C9orf72, has been evident.

We have analyzed 10 unrelated Greek sporadic ALS patients by whole genome sequencing. Our bioinformatics analysis revealed a strong correlation between several single nucleotide polymorphisms (SNPs) with the development of ALS. Subsequently, our analysis was expanded in 27 sporadic ALS patients of Greek origin and 50 ethnically matched healthy controls, which were genotyped by Sanger sequencing and allele-specific PCR.

Our data indicate a statistically significant outcome ( $p < 0.001$ ) for one of the studied SNPs when ALS patients are compared to healthy individuals.

As far as we know, this is the first study that reveals such an association between the FTO gene and the pathobiology of the disease. Nevertheless, due to small sample size, our study should only be considered a pilot study and replication in a larger population cohort is needed to confirm this finding.

**P09.015****ANKK1 locus and Parkinson's disease risk**

J. Hoenicka<sup>1</sup>, D. Martínez-Rubio<sup>2</sup>, E. Pérez-Santamarina<sup>2</sup>, P. García-Ruiz<sup>3</sup>, I. Pla<sup>4</sup>, M. Ezquerro<sup>5</sup>, M. Martí<sup>6</sup>, J. Puente<sup>6</sup>, F. Palau<sup>1</sup>;

<sup>1</sup>Institut de Recerca Pediátrica, Hospital Sant Joan de Déu, Barcelona, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain,

<sup>3</sup>Fundación Jiménez Díaz, Madrid, Spain, <sup>4</sup>Centro de Investigación Príncipe Felipe, Valencia, Spain, <sup>5</sup>Hospital Clínic de Barcelona, Barcelona, Spain, <sup>6</sup>LabGenetics, Madrid, Spain.

**Introduction:** Single nucleotide variations (SNVs) of the ANKK1 gene have been reported to be associated with a wide spectrum of dopamine-related neuropsychiatric and cognitive traits. Given that cerebral dopamine depletion is a hallmark of Parkinson disease (PD) here we hypothesized that ANKK1 regulatory SNVs contribute to PD risk.

**Materials and Methods:** The study included a population sample of 200 PD patients (age 69.48±9.51 years) and 300 healthy controls (age 60.03±3.33) from the BancoADN, the DNA National Bank Carlos III in Spain, and 288 PD patients (age 53.40±11.91) from the clinical series of Hospital Clínico de Barcelona (HCB). ANKK1 regulatory regions were studied using ENCODE data and Haploview and JASPAR software. Genotyping was performed by Tfl restriction digestion of PCR-amplified fragments. Functional validation was assessed by Luciferase Assay and Electrophoretic Mobility Shift Assay. **Results:** Our *in silico* analysis showed that rs7107223 could be a functional SNV that modulates transcription in the ANKK1 locus. It is located at the DNase I hypersensitive site (DHS) at the 5'ANKK1 gene. Genotyping of rs7107223 (Adenine [A] or Thymine [T]) SNV in the Spanish PD population showed the significant overrepresentation of A allele (P=0.0008) and genotypes following a dominant model (P=0.003; OR: 1.831). These findings were replicated in the HCB clinical sample. Functional validation studies indicated significant differences between A and T alleles in the binding, the promotor activity and the response to apomorphine.

**Conclusion:** rs7107223 is a regulatory variant of ANKK1 locus associated with PD risk.

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**P09.016****Protective effects of ApoE ε2 on cognitive performance are gender-specific in healthy elderly from the AsIA-Neuropsychology Study**

M. Vía<sup>1,2</sup>, L. Prades-Senovilla<sup>1</sup>, N. Lamona<sup>1</sup>, J. López-Olóriz<sup>1</sup>, E. López-Cancio<sup>2</sup>, J. F. Arenillas<sup>3</sup>, M. T. Alzamora<sup>4</sup>, G. Pera<sup>5</sup>, P. Torán<sup>5</sup>, I. C. Clemente<sup>1,2</sup>, A. Dávalos<sup>3</sup>, M. Mataró<sup>1,2</sup>,

<sup>1</sup>Dept. Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain,

<sup>2</sup>Institute of Neurosciences, University of Barcelona, Barcelona, Spain, <sup>3</sup>Department

of Neurosciences, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain, <sup>4</sup>Stroke Unit, Department of Neurology, Hospital

Clinico Universitario, Valladolid, Spain, <sup>5</sup>Primary Healthcare Research Support Unit

Metropolitana Nord, IDIAP Jordi Gol, Santa Coloma de Gramenet, Spain.

**Background:** Age-associated cognitive decline and dementias have a substantial hereditary component but the effects of genetic risk variants may be mediated by individual characteristics. We analyzed potential associations of allelic variants in genes involved in neuronal growth and synaptic plasticity with cognitive performance in a large dementia-free elder population and explored potential interactions with age and gender.

**Methods:** The AsIA-Neuropsychology Study included 747 Spanish subjects older than 50 (mean age=66.1±7.6) with a moderate-high vascular risk (assessed by REGICOR score) and free of symptomatic vascular disease or dementia. Markers (SNPs) in 5 genes (APOE, BDNF, GCSF, VEGFA, and SDF1a) were genotyped. Neuropsychological assessment included tests in three cognitive domains: visuospatial skills and speed, verbal memory, and verbal fluency. Linear regression models were controlled for gender, age, years of schooling, and metabolic syndrome.

**Results:** ApoE ε2 allele was associated with a better performance in the memory and fluency domains (p=3•10-4 and p=0.01, respectively). A significant interaction with gender revealed that these associations were present only in women (p=2.87•10-5 and p=0.0023, respectively) and not in men, and were replicated for every individual neuropsychological test included in those domains. No other relevant associations or interactions were observed.

**Conclusions:** Our results show a strong effect of the ApoE ε2 allele in women in different neuropsychological assessments for both verbal memory and fluency, suggesting a high protective effect. The ApoE ε4 allele did not have a substantial effect on cognition in our cohort.

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**P09.017****Contribution of chromosomal aberrations in mosaicism to Autism Spectrum Disorders**

M. Lopez-Sánchez<sup>1,2,3</sup>, I. Cuscó<sup>1,2,4</sup>, A. Cáceres<sup>3,5</sup>, J. R. Gonzalez<sup>2,5</sup>, L. A. Pérez-Jurado<sup>1,2,4</sup>,

<sup>1</sup>Genetics Unit, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>3</sup>Centre de Recerca en Epidemiología Ambiental (CREAL), Barcelona, Spain, <sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain, <sup>5</sup>Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

**Introduction:** Autism Spectrum Disorder (ASD) is one of the most frequent neurodevelopmental disorders. ASD has a strong genetic component in its etiology, including de novo germ-line mutations and copy number variants. Detectable mosaicism for chromosomal rearrangements in blood has been reported in >1% of the aging population (>65y), but is very rare in young people. Chromosomal mosaicism due to early developmental events could be causative of ASD.

**Methods:** We have studied molecular karyotypes (SNP array) of blood DNA of two large ASD datasets, the Autism Genome Project and the Simons Simplex Collection (4427 patients, 9268 parents, 2433 unaffected siblings) for mosaic chromosomal alterations >0.4Mb, using the MAD and Tripod softwares. As age-matched controls, we also used reported data of 5094 children with no developmental abnormalities. We independently analyzed data from cell-line DNA of 564 patients and 806 parents.

**Results:** Mosaic alterations (0.4-155Mb) were detected in blood DNA of 21 out of 4427 patients (0.47%). A total of 34 events were also detected in parental samples (0.37%) and 5/7517 unaffected children (0.07%). The frequency of detectable mosaicism is significantly higher in ASD patients. In cell-line DNA, the frequency was similar in cases and parents although 6 unbalanced chromosomal translocations were detected in patients and just 1 in parents.

**Conclusion:** Chromosomal aberrations present in mosaicism are detected in blood samples of a small but significant proportion of children with ASD (0.47%). This finding at early ages suggests that mosaicism may be present in other cell types affecting brain development and causing ASD.

**P09.018****Genomic and genetic variation at complex segmental duplications in Autism Spectrum disorders**

M. Codina-Solà<sup>1,2,3</sup>, R. Flores<sup>1,2,3</sup>, A. Homs<sup>1,2,3</sup>, C. Aguado<sup>1,2,3</sup>, L. Pérez-Jurado<sup>1,2,3</sup>, I. Cuscó<sup>1,2,3</sup>,

<sup>1</sup>Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Neurosciences Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Barcelona, Spain.

**Introduction:** Autism Spectrum Disorders (ASD) are among the most heritable neurodevelopmental conditions but the aetiology remains elusive in a high proportion of cases. Complex rearrangements and gene conversion events in segmental duplications (SDs) could contribute to the phenotype, but are largely unstudied. To determine their variation and role in ASD, we have studied two candidate hot-spot regions: the Williams-Beuren syndrome locus at 7q11.23 and the chr9 pericentromeric region.

**Methods:** We performed targeted sequencing of the multi-copy genes in SDs (*GTF2I*, *GTF2IRD2* and *CNTNAP3*) and single-copy genes in 279 ASD patients and 105 controls. To identify CNVs, SNVs and gene conversions, we mapped the sequences to a single paralogous copy and integrated read depth and relative paralogous sequence quantification. All results were experimentally validated by other methods in an extended case and control population.

**Results:** Copy-gains of a 7q11.23 block containing truncated *GTF2I* and functional *GTF2IRD2* genes were identified in 1.8% ASD cases vs 0.45% controls. We also identified a disruptive mutation (p.M1?) in a *GTF2IRD2* copy in two affected brothers, inherited from their father. In the chr9 pericentromeric region, a *de novo deletion* was identified in a patient but the global distribution of rearrangements and rare SNVs did not differ significantly between controls and patients.

**Conclusions:** Despite their complex genetic architecture, our method successfully identifies rearrangements and SNVs in SDs, such as copy-gains and mutations at *GTF2IRD2* or deletions of *CNTNAP3* that could represent susceptibility factors for ASD.

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## P09.019

**The power of New Generation Sequencing in identifying mutations in non-specific ASD-ID phenotypes: the example of SHANK3**

**A. Mosca-Boidron**<sup>1,2</sup>, H. Poquet<sup>2</sup>, M. Willems<sup>3</sup>, J. Thevenon<sup>4,2</sup>, C. Redin<sup>5</sup>, C. Thauvin<sup>4,2</sup>, Y. Duffourd<sup>2</sup>, S. Lumbroso<sup>6</sup>, B. Gérard<sup>6</sup>, J. Mandel<sup>6</sup>, F. Amsellem<sup>7</sup>, A. Beggiato<sup>7</sup>, R. Delorme<sup>7</sup>, N. Marle<sup>1</sup>, P. Callier<sup>1,2</sup>, J. Rivière<sup>2</sup>, P. Kuentz<sup>2</sup>, A. Piton<sup>5</sup>, L. Faivre<sup>4,2</sup>,

<sup>1</sup>Cytogenetics, Dijon, France, <sup>2</sup>Equipe Génétique et Anomalies du Développement, Dijon, France, <sup>3</sup>Département de Génétique médicale, Montpellier, France, <sup>4</sup>Département de Génétique médicale, Dijon, France, <sup>5</sup>IGBMC, Strasbourg, France, <sup>6</sup>Laboratoire de Biochimie, Nîmes, France, <sup>7</sup>Psychiatrie de l'enfance et de l'adolescence, Hôpital Robert-Debré, Paris, France.

**Introduction:** Autism spectrum disorders (ASDs) are characterized by impairments in reciprocal social communication and stereotyped behaviors. Intellectual Disability (ID) frequently coexists with these two core symptoms. Monogenic causes are rare, among them, de novo or truncating mutations in *SHANK3* concern almost one in 50 children with autism and moderate to profound ID. *SHANK3* haploinsufficiency is frequently associated with neonatal hypotonia, moderate to severe ID, absent to severely delayed speech, ASD and moderate dysmorphic features (Phelan-McDermid syndrome). Thus, the question arises to look for the presence of distinctive signs to identify a subgroup of individuals who could benefit from the sequencing of this gene.

**Materials and Methods:** Here we report on 2 boys with ASD and ID and a girl with severe ID associated with a *SHANK3* mutation found by next generation sequencing (NGS) study (Illumina).

**Results:** Two of these mutations are truncated and de novo involving the exon 21 in the prolin-rich domain (NM\_001080420.1:c.2955\_2970dup (p.(Pro992Arg\*325) and NM\_001080420.1:c.4381C>T (p.(Gln1461\*)). The third is a 3 bases deletion variant NM\_001080420.1:c.5090\_5092delACC (p.(His1197del)) involving the exon 22 in the SAM (Sterile-Alpha-Motif) domain involved in multimerization of *SHANK3* protein.

**Conclusion:** The retrospective clinical examination of these patients did not permit to distinct specific signs, which highlights the phenotypic heterogeneity of these patients. Given the scarcity of variants involving *SHANK3*, the unsystematic presence of the distinctive clinical features of Phelan McDermid Syndrome, a NGS approach by panel or exome seems more justified than a targeted sequencing approach of *SHANK3* in this phenotype.

## P09.020

**Next generation sequencing approaches in autosomal dominant cerebellar ataxias: from panel cohort study to new gene identification**

**M. Coutelier**<sup>1,2</sup>, I. Blesneac<sup>3</sup>, G. Coarelli<sup>1</sup>, A. Montell<sup>3</sup>, M. Monin<sup>1</sup>, K. Ando<sup>4</sup>, E. Mundwiller<sup>5</sup>, A. Brusco<sup>6</sup>, I. Le Ber<sup>1</sup>, M. Anheim<sup>7</sup>, A. Castrioto<sup>8</sup>, C. Goizet<sup>9</sup>, C. Duyckaerts<sup>1</sup>, P. Lory<sup>9</sup>, A. Brice<sup>1</sup>, A. Durr<sup>1</sup>, G. Stevanin<sup>1</sup>;

<sup>1</sup>INSERM U1127, Institut du Cerveau et de la Moelle Epinière, Paris, France, <sup>2</sup>Laboratory of Human Molecular Genetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium, <sup>3</sup>Centre National de la Recherche Scientifique UMR 5203 and INSERM U 1191, Institut de Génomique Fonctionnelle, Université de Montpellier, Montpellier, France, <sup>4</sup>Centre de Référence de Neurogénétique, Hôpital de la Pitié-Salpêtrière, Assistance Publique – Hôpitaux de Paris, Paris, France, <sup>5</sup>Plateforme de Génotypage et Séquençage, Institut du Cerveau et de la Moelle Epinière, Paris, France, <sup>6</sup>Department of Medical Sciences, University of Turin, Medical Genetics Unit, Città della Salute e della Scienza University Hospital, Torino, Italy, <sup>7</sup>Département de Neurologie, Hôpital de Hautepierre, Centre Hospitalier Universitaire de Strasbourg, Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM U964, Centre National de la Recherche Scientifique UMR7104, Université de Strasbourg, Strasbourg, France, <sup>8</sup>Unité Troubles du Mouvement, Pôle de Neurologie et Psychiatrie, Centre Hospitalier Universitaire de Grenoble, Grenoble, France, <sup>9</sup>Université de Bordeaux, Laboratoire Maladies Rares : Génétique et Métabolisme, EA4576, Bordeaux, France.

Autosomal dominant cerebellar ataxias (ADCA) are heterogeneous neurodegenerative diseases, associating a cerebellar syndrome to various other manifestations. They are frequently caused by polyglutamine expansions. Intronic repeat expansions, and conventional mutations, come next. Though 30 genes have been identified, the causative mutation is still unknown in 40% of patients.

We explored ADCA aetiologies, combining whole genome linkage analysis and exome sequencing in large families, to amplicon panel sequencing of known and candidate genes in 412 patients.

Sequencing a large cohort allowed us to obtain valuable information regarding the not much known nosology of conventional ADCA. We identified 17 patients with convincing single nucleotide variants in *CACNA1A* (4.12%). These are classically associated to episodic ataxia 2, while small nucleotide expansions are linked to progressive ataxia. We hereby confirm that these presentations are not genetically different. We also identified eight patients with biallelic mutations in *SPG7*; as well as eight carriers of p.Ala510Val alone, whose pathogenicity we discuss.

In this study, we also identified new candidate genes. *CACNA1G* encodes T-type Calcium channel Cav3.1. A variant of its voltage-sensing domain,

p.Arg1715His, recurrent in three pedigrees, has consequences on the electrophysiological characteristics of the channel, driving lowered excitability. This allows an interesting link with epilepsy, where *CACNA1G* gain-of-function variants are risk factors.

We hence report an epidemiological study of an unprecedentedly large ADCA cohort, which has direct clinical consequences; and a new causative gene, confirming the prominence of ion channels in ADCA pathophysiology. This work was funded by the European Union, F.R.S.-FRNS, ANR and VERUM.

## P09.021

**Spinocerebellar ataxia type 37 (SCA37): first neuropathological findings and molecular characterisation of the critical region on 1p32**

**M. Corral-Juan**<sup>1</sup>, C. Serrano-Munera<sup>2</sup>, A. Rábano<sup>3</sup>, E. Gelpi<sup>4</sup>, L. Saiz<sup>2</sup>, M. Del Pozo-Valero<sup>1</sup>, L. Isprierto<sup>5</sup>, R. Álvarez<sup>5</sup>, V. Volpini<sup>6</sup>, I. Sánchez<sup>7</sup>, A. Matilla-Dueñas<sup>1</sup>;

<sup>1</sup>Health Research Institute Germans Trias-ICTP, Badalona, Spain, <sup>2</sup>Hospital Sant Joan de Déu de Martorell, Martorell, Spain, <sup>3</sup>CIEN Foundation, Madrid, Spain, <sup>4</sup>Neurological Tissue Bank of the Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain, <sup>5</sup>Hospital Universitari Germans Trias, Badalona, Spain, <sup>6</sup>IDIBELL, Hospitalet de Llobregat, Spain.

**Introduction:** We recently reported a new ataxia subtype, SCA37, characterised by slow progressing ataxia and altered vertical eye movements in 2 Spanish kindreds. Here we report the first neuropathological findings and the studies aimed at identifying the SCA37 molecular deficit on 1p32.

**Material and Methods:** Neuropathology, WGS, and custom-aCGH studies were performed. Cerebellum-specific RACE and Purkinje cells (PCs) RNAseq data from ENCODE were valuable to characterise putative cerebellar SCA37 transcripts. The SCA37 critical region was refined with WGS, SNPs, microsatellites, and long-PCR sequencing.

**Results:** Marked neuronal loss was present in the cerebellar cortex and inferior olives. Extensive Bergmann gliosis and loss of calbindin immunoreactivity, with aberrant dendrite arborisation, nuclear lobulation, irregularity and hyperchromatism, and multiple small perisomatic ubiquitinated inclusions were identified in cerebellar PCs. By WGS and genetic analyses, the SCA37 critical region was narrowed to 1.742 Mb on 1p32. Two non-recombinant SCA37-linked SNPs were useful to identify additional SCA37 index cases. Cerebellar RACE and PC RNAseq studies identified new putative regulatory exons within the SCA37 transcript selectively expressed in Purkinje cells. Long-PCR sequencing for SNP-allele discrimination identified putative structural regulatory candidate mutations in SCA37.

**Conclusions:** We report the first neuropathological findings showing diffuse cortical cerebellar degeneration in SCA37. Two linked-SNPs prove useful to identify additional SCA37 cases. RACE and RNAseq studies reveal expression of a specific SCA37 transcript in Purkinje cells. We propose that a regulatory structural mutation altering the proper temporal and/or spatial expression of the SCA37 gene underlies cerebellar neurodegeneration in SCA37. Project funded by Spanish ISCIII.

## P09.022

**Rare copy-number variations are associated with specific clinical manifestations in children with autism spectrum disorder**

**I. Shtein, I. Menashe;**

*Ben-Gurion University of the Negev, Beer Sheva, Israel.*

**Introduction:** Autism spectrum disorder (ASD) is a complex heritable neurodevelopmental condition characterized by social, and communication disabilities. However, children with ASD manifest additional symptoms that further complicate the clinical picture of this disorder. Rare copy number variations (CNVs) play a significant role in ASD susceptibility. Here, we ask whether rare CNVs are associated with specific clinical manifestations among children with ASD.

**Methods:** We studied seventy clinical characteristics in 1115 children that are diagnosed with ASD and have CNV data from the Simons Simplex Collection (SSC). Associations between these characteristics and total burden of CNVs as well as specific CNVs, were determined at P<0.1.

**Results:** Rare CNVs were more prevalent among African-American females. Interestingly, core ASD traits (i.e. communication and social difficulties) were associated with genomic burden of duplications whereas severity of clinical comorbidities (e.g. IQ, irritability, and pregnancy optimality scores) were associated with genomic burden of deletions. Further, we found associations between several clinical characteristics and specific ASD-susceptibility CNV loci. Specifically, duplications in 15q11.2-q13.1, and in 16p11.2 were associated with a higher irritability, duplications in 22q11.21 were associated with a worse neonatal optimality score, and CNVs in 9p24.3 were associated with worse verbal ability.

**Conclusions:** Our results suggests that rare CNVs contributes to the severity of ASD across several clinical and behavioral aspects.

**P09.023****Comparison of CRISPR-based methods for modeling loss-of-function in iPSC cells**C. M. Seabra<sup>1,2,3</sup>, P. Manavalan<sup>1</sup>, D. J. C. Tai<sup>1,3</sup>, M. Talkowski<sup>1,3,4</sup>, J. F. Gusella<sup>1,3,4</sup>,<sup>1</sup>Center for Human Genetic Research, Boston, MA, United States, <sup>2</sup>GABBA Program - Institute of Biomedical Sciences Abel Salazar of the University of Porto, Porto, Portugal,<sup>3</sup>Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>4</sup>Departments of Genetics and Neurology, Harvard Medical School, Boston, MA, United States.

**Introduction:** Given the rapid pace of discoveries that de novo loss-of-function (LoF) mutations in highly conserved genes represent penetrant sources of genetic risk in autism spectrum disorder (ASD), it is imperative to generate robust models that recreate the human cellular landscape, particularly for neurological disorders where brain tissue is not readily available, and to eliminate the confound of different genetic backgrounds.

**Methods:** We used an induced pluripotent stem cell (iPSC) line from a healthy male subject to perform dual-guide CRISPR/Cas9 gene editing of 9 independent genes for which LoF mutations represent strong risk factors for ASD. The efficiency of 30 dual guide-RNA combinations to generate deletions was then compared, using either FACS sorting or puromycin selection followed by serial dilutions to obtain single-cell derived colonies.

**Results:** Dual guide CRISPR successfully generated deletions in all genes, however the efficiency varied widely by guide-RNA and cellular protocol. The overall efficiency of the FACS method was 3.4% to generate predicted ablations, out of 1002 colonies screened (range = 0% - 10.6%). By contrast, the puromycin method had an average efficiency of 15.1% from 4437 colonies (range = 0% - 32%).

**Conclusions:** This systematic survey of genome editing approaches suggests that dual-guide deletion generation varies widely by guide-pair. We also find that the increased certainty of deriving a single cell from FACS sorting comes at a significant cost in terms of efficiency and cell viability compared to serial dilutions.

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**P09.024****Resilience formation against chronic stress: the Tsc2KO mouse model to study the involvement of the mTOR pathway**A. Art<sup>1</sup>, J. Winter<sup>1</sup>, S. Schweiger<sup>1</sup>,<sup>1</sup>Institute of Human Genetics, University Medical Center, Johannes Gutenberg University, Mainz, Germany.

Characteristic for autistic children is their reliability on well-structured days and predictable procedures. Deviations of these cause stress in them and result in behavioural abnormalities. We therefore hypothesize that autistic children have reduced resilience for chronic stress. In order to analyse mechanism of stress resilience in autism we are using a Tsc2KO mouse model for tuberous sclerosis. TSC2 (together with TSC1) is part of a complex that inhibits the mechanistic target of rapamycin (mTOR) kinase. Mutations in either of the two genes result in increased mTOR activity and upregulated downstream signalling. mTOR signalling plays an important role in memory formation. It also is supposed to be a key player in stress resilience.

According to these studies we have established a behaviour battery to analyse the consequences of chronic social defeat in Tsc2KO animals. After a 14-days' period of chronic stress exposure mice are being analysed in this battery, which consists of an object recognition test, an elevated plus-maze test, a test of nest building, an evaluation of social interaction, sucrose preference, spontaneous alternation in a Y-maze test, a tail suspension test and the analysis of prepulse inhibition of startle response.

First results show that non-stressed Tsc2KO animals have alterations in social behaviour and in nest building when compared to wildtype mice. Of note, they also show an altered response to stress and changes in stress resilience. These data will give us closer insight into specific problems in autism and will also gain our understanding of mechanisms underlying stress resilience.

**P09.025****Association between copy-number variations and savant skills among people with autism spectrum disorder**H. Rosenthal<sup>1</sup>, I. Menashe<sup>1</sup>,<sup>1</sup>Ben-Gurion University of the Negev, Beer Sheva, Israel.

**Background:** Savant skills, are reported in 10%-30% of people with autism spectrum disorder (ASD). The co-existence of these two traits suggests common underlying mechanisms that require further exploration. Copy-number

variations (CNVs) are excellent candidate to mutually underlie these two distinct conditions.

**Methods:** We studied the association between CNVs and savant skills among 1108 children diagnosed with ASD from the Simons Simplex Collection (SSC) database. Savant skills were determined based on five designated questions from the autism diagnostic interview revised (ADI-R) questionnaire. CNVs data were retrieved from a genome-wide analysis of CNVs in these children. **Results:** Savant skills were significantly more prevalent in our sample than in other children with ASD in the SSC cohort (42% vs. 29%; P<0.001). Children with savant skills had, on average, a higher IQ (93.0±24.2 vs. 79.1±25.5; P<0.001), and a larger head circumference (HC) (54.3±2.55 vs. 53.8±2.60; P = 0.003). Interestingly, savant skills were associated with a lower count of rare CNVs among children with ASD (14.0±5.54 vs. 14.8±6.2 for children with and without savant skills respectively; P = 0.03). In addition, children with exceptional computational ability had lower burden of rare deletions compared to other children (203.46±164.25kb vs. 333.67±715.56kb; P = 6.8x10-5) even after accounting for IQ and socioeconomic status. We also examined whether certain talents are predisposed by specific ASD susceptibility CNV loci, but no such associations were found.

**Conclusions:** Our findings suggest that CNVs contribute to the presentation of exceptional talents among children with ASD.

**P09.026****Peculiar brain activity in autism spectrum disease due to 11p15.4-15.5 duplication**M. Szegedi<sup>1</sup>, G. Inczédy-Farkas<sup>1</sup>, Á. Szabó<sup>2</sup>, I. Haltrich<sup>3</sup>, G. Fekete<sup>3</sup>, G. Rudas<sup>2</sup>, L. R. Kozák<sup>2</sup>,M. J. Molnár<sup>1</sup>,<sup>1</sup>Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary, <sup>2</sup>MR Research Centre - Szentágothai Knowledge Center, Semmelweis University, Budapest, Hungary, <sup>3</sup>2nd Department of Paediatrics, Semmelweis University, Budapest, Hungary.

**Introduction:** ASD is a neurodevelopmental disorder with multiple genetic and non-genetic causes. The 11p15.4-15.5 duplication can be associated with intellectual disability and dysmorphic features. The aim was to assess the neural networks involved in visual and verbal memory encoding and retrieval in ASD due to 11p15.4-15.5 duplication.

**Materials and methods:** A woman with autistic features, macrocephaly and body asymmetry due to 11p15.4-15.5 duplication and 7 healthy controls were investigated. Six runs of BOLD fMRI data were collected to assess the networks of the visual and verbal memory encoding and retrieval; a single run of encoding task was followed by 10 minute-retention period, then 2 runs of the retrieval tasks both for visual and verbal domain respectively. During encoding participants were instructed to memorize the task stimuli, whilst during retrieval they were instructed to decide whether the given image or word was presented during the encoding period. Data analysis was performed using the SPM8 toolbox under Matlab, with standard processing steps.

**Results:** In patient fMRI for visual memory encoding/retrieval showed higher activation in the posterior insulae, and anterior and posterior cingulate cortices compared to the controls where visual areas were predominant in these tasks. Verbal memory activation was higher in the hippocampi, parahippocampal cortices, precuneus; and lower in the anterior insulae; higher visual areas in our patient compared to the controls.

**Conclusion:** The 11p15.4-15.5 duplication resulted in peculiar activity pattern of the brain responsible for visual and verbal memory in ASD. This observation may help to understand the pathogenesis of ASD.

**P09.027****Association and functional significance of SNPs in the AVPR1A gene in autism spectrum disorder in Korean population**H. Yoo<sup>1,2</sup>, S. Kim<sup>3</sup>, S. Yang<sup>3</sup>, J. Park<sup>4</sup>, M. Park<sup>3</sup>,<sup>1</sup>Seoul National University Bundang Hospital, Seongnam, Korea, Republic of, <sup>2</sup>Seoul National University College of Medicine, Seoul, Korea, Republic of, <sup>3</sup>Eulji University, Daejon, Korea, Republic of, <sup>4</sup>National Institute of Animal Science, Wanju, Korea, Republic of.

**Objectives:** The arginine vasopressin receptor 1A gene (AVPR1A) is related to social reciprocity in humans and animals. The objective of this study is to evaluate the association of AVPR1A with autism spectrum disorder (ASD) and the functional significance of the markers.

**Methods:** 1) The probands with ASD and their biological parents were recruited. Diagnosis was ascertained using ADI-R and ADOS. 2) Two microsatellites (RS3, RS1) in the 5' flanking region and 2 SNPs in the promoter region of AVPR1A were genotyped. Transmission disequilibrium test and quantitative association test with behavior measures were performed using the FBAT package (v.2.0.2c). 4) For evaluation of the functional significance of the associated marker, luciferase assay was performed.

**Results:** Total 212 family trios (9 multiplex families, 644 persons) participated. 1) One SNP (rs10877969) was strongly associated with ASD (additive p-value=1.62x10-6; dominant p-value=4.81x10-6). Haplotypes with rs10877969 and rs72945336 revealed statistical significances at the multiallelic mode (additive p-value=2.2x10-5; dominant p-value=1.43x10-5). 2) The rs10877969 was quantitatively associated with Social Responsiveness Scale and all subdomain scores of ADI-R ( $p < 0.01$ ). 3) In the luciferase assay with T98G cell line, the luciferase activity of rs7294536A promoter was higher than that of rs7294536G, while rs10877969 allelic variants didn't influence to promoter activity.

**Conclusion:** We observed significant association of an SNP of AVPR1A with affection status and social phenotypes of ASD, accompanied with functional activity of the marker.

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#### P09.028

#### Copy number variation in 19 Italian multiplex families with autism spectrum disorder: importance of synaptic and neurite elongation genes

*C. Picinelli<sup>1</sup>, C. Lintas<sup>2</sup>, I. Piras<sup>1</sup>, R. Sacco<sup>2</sup>, C. Brogna<sup>2</sup>, A. M. Persico<sup>1,3</sup>,*

<sup>1</sup>*Mafalda Luce Center for Pervasive Developmental Disorders, Milan, Italy, <sup>2</sup>Unit of Child and Adolescent NeuroPsychiatry & Laboratory of Molecular Psychiatry and Neurogenetics, University „Campus Bio-Medico”, Rome, Italy, <sup>3</sup>Unit of Child and Adolescent Neuropsychiatry, “Gaetano Martino” University Hospital, University of Messina, Messina, Italy.*

Autism Spectrum Disorder is endowed with impressive heritability estimates and high recurrence rates. Its genetic underpinnings are very heterogeneous and include many common and rare variants located in hundreds of different loci, each characterized by variable levels of penetrance. Multiplex families from single ethnic groups represent a useful means to reduce heterogeneity and enhance genetic load. We screened 19 Italian ASD multiplex families (3 triplets and 16 duplets, total N=41 ASD subjects), using array-CGH (Agilent 180K). Certainly or probably causal CNVs, defined “clinically relevant CNVs”, were detected in 17/41 (41%) of ASD probands, corresponding to 9/19 (47%) multiplex families with at least one affected sibling genetically positive. However only in 3/9 (33%) of these families, siblings share the same causal or highly causal CNV. Additional potentially relevant CNVs not shared by affected sib pairs were detected also in these three families. 45 genes are located on the “clinically relevant” CNVs. Through an enrichment analysis, we found that 9/45 (20%) of these genes appear primarily involved in neurite outgrowth and synapse formation/management. Our results highlight the importance of synaptic and neurite elongation genes in the pathogenesis of autism, despite genetic heterogeneity in ASD even within multiplex families belonging to a single ethnic group. Differences in CNV burden may likely contribute to the substantial clinical heterogeneity observed between affected sibs. The genetic and epigenetic mechanisms underlying genomic instability in these families deserve further scrutiny.

#### P09.029

#### Increased frequency of the autism broader phenotype in mothers transmitting etiological CNVs to sons affected by Autism Spectrum Disorder (ASD)

*M. Asif<sup>1,2</sup>, I. Conceição<sup>3,2</sup>, K. Kwiatkowska<sup>2</sup>, C. Rasga<sup>3,2</sup>, C. Café<sup>4</sup>, L. Sousa<sup>5</sup>, G. Oliveira<sup>4,6</sup>, F. M. Couto<sup>7</sup>, A. M. Vicente<sup>3,2,8</sup>,*

<sup>1</sup>*Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, <sup>2</sup>Biosystems and Integrative Sciences Institute, Lisbon, Portugal, <sup>3</sup>Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal, <sup>4</sup>Unidade de Neurodesenvolvimento e Autismo do Serviço do Centro de Desenvolvimento da Criança e Centro de Investigação e Formação Clínica, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, <sup>5</sup>University of Lisbon, Faculty of Sciences, DEIO and CEUAL, Lisbon, Portugal, <sup>6</sup>University Clinic of Pediatrics and Institute for Biomedical Imaging and Life Science, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>7</sup>Department of Informatics, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, <sup>8</sup>Instituto Gulbenkian de Ciência, Oeiras, Lisbon, Portugal.*

Autism Spectrum Disorder is a frequent neurodevelopmental disorder with a high male to female ratio. An increased prevalence of autism-like personality traits is found in unaffected relatives of ASD children, suggesting a genetic liability of a broader autism phenotype. We therefore hypothesized that the parents of ASD children who transmit etiological CNVs might exhibit ASD traits more frequently than non-transmitting parents. To test this hypothesis, we analysed CNV inheritance and parental behavioral traits in families from the Autism Genome Project, assessed using the Broad Autism Phenotype Questionnaire (BAPQ) (N=341) and the Social Responsiveness Scale (SRS) (N=456). We selected CNVs spanning well-established candidate

genes for ASD, and compared transmitting and non-transmitting parental test scores using a t-test corrected for multiple testing by the Group Benjamini-Hochberg Procedure.

Overall, CNV-transmitting parents did not differ significantly in BAPQ and SRS scores from non-transmitting parents. However, independent analyses of relative pairs revealed a significant difference in BAPQ global ( $t=-2.18$ ; adjusted  $P=.032$ ), BAPQ aloofness domain ( $t=-2.61$ ; adjusted  $P=.032$ ) and SRS scores ( $t=-2.03$ ; adjusted  $P=.047$ ) between mothers transmitting and mothers not transmitting etiological CNVs to their affected sons. Our findings indicate that mothers presenting personality traits in the broader autism phenotype are frequently carriers of pathogenic CNVs that they transmit to their ASD sons. The results from the analyses of maternal phenotype and CNV transmission patterns to sons support previous reports of maternal transmission bias to male offspring, and the prevalent hypothesis of a higher genetic risk tolerance in females due to putative protective factors. (FCT PD/BD/52485/2014)

#### P09.030

#### AUTS2 syndrome: Further delineation of the phenotype in a 68-years-old female

*E. Sengun<sup>1</sup>, K. Yararbas<sup>2,3</sup>, Y. Alanay<sup>1</sup>,*

<sup>1</sup>*Acibadem University School of Medicine, Istanbul, Turkey, <sup>2</sup>Maltepe University School of Medicine, Istanbul, Turkey, <sup>3</sup>Duzen Laboratories Group, Istanbul, Turkey.*

**Introduction:** Genetic evaluation of individuals with neurodevelopmental disorders, with technical improvements in array based technologies and sequencing, has yielded an abundance of new candidate genes for intellectual disability (ID), autism spectrum disorders (ASDs), and developmental delay. We report a 68 year old female with mild-moderate intellectual disability, behavioral findings suggesting ASD, developmental delay and dysmorphic features. The SNP array analysis demonstrated a 257 kb deletion comprising exon 6 of AUTS2 gene.

**Case Report:** The patient was born at term as the first child of an unrelated couple following an uncomplicated pregnancy and delivery. Mild motor and significant speech delay were evident during childhood. On her physical examination at age 68, she had short stature, microcephaly and abdominal obesity. Mild facial findings and micrognathia were noted. She was followed for scoliosis since early adulthood. Orthopedic findings were present. Her intellectual disability was mild to moderate with behavioral problems. She was noted to be a very friendly, active and girly person. She had limited eye-contact, hyperverbalism with limited vocabulary of 40-50 words. She had tics and obsessions, skin prickling, hyperorality and sound-sensitivity. She was not schooled but her family made sure that she was involved in daily activities.

**Conclusions:** This clinical report provides the natural history in the eldest patients yet to be reported and complements the existing evidence suggesting that disruption of the AUTS2 gene leads to a recently delineated neurodevelopmental phenotype with a wide spectrum, namely “AUTS2 Syndrome”.

#### P09.031

#### Targeted next-generation sequencing in search of monogenic causes of behavioural disturbance in children

*K. Komlósí<sup>1</sup>, S. Diederich<sup>1</sup>, D. L. Fend-Guella<sup>1</sup>, O. Bartsch<sup>1</sup>, H. Hu<sup>2</sup>, T. F. Wienker<sup>2</sup>, H. H. Ropers<sup>1,2</sup>, M. Huss<sup>3</sup>, J. Stewe<sup>3</sup>, U. Zechner<sup>4</sup>, S. Schweiger<sup>5</sup>,*

<sup>1</sup>*Institute of Human Genetics, University Medical Center, Johannes Gutenberg University Mainz, Mainz, Germany, <sup>2</sup>Max-Planck Institute for Molecular Genetics, Berlin, Berlin, Germany, <sup>3</sup>Center for Pediatric Psychiatry, Psychotherapy and Psychosomatic at the Rheinhessen Clinic Mainz, Mainz, Germany.*

**Introduction:** Behavioral disturbance can be the presenting symptom of genetically determined cognitive deficiency caused by de novo dominant, rare recessive and pathogenic copy number variations (CNV). NGS has accelerated diagnosis in genetically heterogeneous disorders with common clinical features.

**Patients and methods:** 28 children with unclear developmental delay and behavioral disturbance seen at a joint clinical genetic and psychiatric outpatient clinic underwent targeted NGS testing including over 1200 brain related genes (MPIMG-1). Following enrichment 2x300bp paired-end sequencing (Illumina MiSeq Kit v3) was carried out on Illumina MiSeq and a modified Medical Resequencing Analysis Pipeline was used for variant calling. **Results:** In 3/28 cases pathogenic or likely pathogenic CNVs, in 8/28 a monogenic cause was identified. We found 6 cases with mutations in autosomal dominant neurodevelopmental genes, either reported and matching the patient's phenotype (PTPN11, SETBP1), or likely associated with the phenotype (DYRK1A, GRIN2B, ASXL1, ZEB2) but previously unreported.

In siblings with absent speech, intellectual disability, aggressive behavior and seizures a homozygous ALG1 mutation was associated with a mild form of CDG1k. In a further 4 patients, likely pathogenic unreported variants in known disease-causing genes (ASXL1, BRCA2, MBD5) were identified. Conclusions: Targeted NGS can be effective in delineating monogenic causes of a common clinical symptom associated with behavioural abnormalities. A definitive diagnosis helps the family come to terms with the condition and enables personalized treatment and care. Subsequent functional characterization of gene products via patient-specific iPSC models may lead to a better understanding of the mechanisms and open up future therapeutic possibilities.

#### P09.032

#### Hyperkinetic movement disorders with pediatric-onset related to ADCY5 gene mutations: report of three Italian families

E. Mantuano<sup>1</sup>, M. Carecchio<sup>2,3,4</sup>, N. E. Mencacci<sup>5</sup>, G. Zorzi<sup>3</sup>, F. Zibordi<sup>3</sup>, A. Iodice<sup>6</sup>, C. Fusco<sup>6</sup>, L. Veneziano<sup>1</sup>, C. Barzaghi<sup>2</sup>, C. Panteghin<sup>2</sup>, L. R'Bilo<sup>5</sup>, N. Wood<sup>5</sup>, B. Garavaglia<sup>2</sup>, N. Nardocci<sup>3</sup>;

<sup>1</sup>Institute of Translational Pharmacology, National Research Council, Rome, Italy,

<sup>2</sup>Molecular Neurogenetics Department, Movement Disorders Diagnostic Unit, IRCCS Foundation C. Besta Neurological Institute, Milan, Italy, <sup>3</sup>Department of Child Neurology, IRCCS Foundation C. Besta Neurological Institute, Milan, Italy, <sup>4</sup>Department of Translational Medicine, University of Milan Bicocca, Milan, Italy, <sup>5</sup>Department of Molecular Neuroscience, Institute of Neurology, University College London, London, United Kingdom, <sup>6</sup>Child Neuropsychiatry Unit, Santa Maria Nuova Hospital, Reggio Emilia, Italy.

Background: ADCY5 is a recently identified gene responsible for a wide spectrum of mixed hyperkinetic early-onset movement disorders including chorea, myoclonus and dystonia. Similarly to benign hereditary chorea due to TITF-1 mutations, the disease course seems to be non-progressive, but severe abrupt diurnal and nocturnal exacerbations of movement disorder are often present. To date, 7 mutations in 21 unrelated dominant families and sporadic cases have been reported.

Methods: 35 Italian unrelated cases with pediatric onset hyperkinetic movement disorder featuring a combination of chorea, myoclonus and dystonia who tested negative for TITF-1 mutations were recruited. ADCY5 exons 2 and 10, in which mutations have been identified in ~86% of families published to date, were sequenced.

Results: 3/35 cases (8.5%) showed mutations in exon 10. Two sporadic cases carried previously reported mutations (p.R418W, p.R418Q) and one familial case with autosomal dominant inheritance carried a novel mutation (p.R418G).

All patients presented between 1 and 4 years of age with delayed milestones and a movement disorders characterized by generalized dyskinesias, myoclonic jerks and mild dystonia. One patient showed prominent pyramidal signs in the lower limbs and perioral dyskinesia. In two cases exacerbations of hyperkinesias at night and during the day without specific triggers were described. In adolescence one patient switched from a choreic to a prominent myoclonic phenotype.

Conclusions: ADCY5 mutations are an important cause of early-onset, mixed hyperkinetic movement disorders. Paroxysmal worsening of movement disorders both during the day and at night seems to be a key diagnostic element in these cases.

#### P09.033

#### Bipolar Related Functional Variants in Calcium Channel Genes

N. L. O'Brien, A. Fiorentino, S. Sharpe, M. Way, M. Morgan, D. Curtis, N. Bass, A. McQuillin;

University College London, London, United Kingdom.

Bipolar Disorder (BD) is a highly heritable psychiatric illness. Members of the calcium channel gene family have been repeatedly implicated in BD through genome wide association studies, case-case meta-analysis and pathway analysis. This gene family includes the genes CACNA1C; encoding the  $\alpha$  pore of L-type calcium channels, and CACNG4; encoding a transmembrane AMPA receptor regulator involved in trafficking AMPA receptors to the neuronal post synaptic density.

High resolution melting curve (HRM) analysis and whole genome sequencing (WGS) methods were used to identify functional variants in these genes in the UCL BD cohort. Variants predicted to impact gene regulation, transcription or to be damaging to protein structure were genotyped in a larger case/control cohort. For rare variants publicly available data, from healthy volunteers and individuals with psychiatric illnesses, was utilised for a more accurate determination of allele frequencies.

HRM identified two rare non-synonymous CACNG4 variants associated with mental illness (rs371128228, p=1.05x10<sup>-4</sup>, OR=4.39 and

17:65026851(C/T), p=5x10<sup>-4</sup>, OR=9.52). Fluorescent activated cell sorting analysis demonstrated that the risk allele of rs371128228 decreased cell surface trafficking of AMPA-R1 (p=0.026).

WGS analysis of CACNA1C intron 3 identified two BD associated (p=0.015, OR=1.15) variants 105bp apart that were in complete LD. Both variants are predicted to create YY1 transcription factor binding sites. Luciferase reporter assays show a significant decrease in gene expression in the presence of both variants (p=0.004).

Using two different approaches this study identified four functional variants in two genes previously associated with BD that have a functional effect *in vitro*.

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#### P09.034

#### Unravelling the genetic basis of bipolar disorder through exome sequencing in extended families with multiple affected relatives

C. Toma<sup>1,2</sup>, A. D. Shaw<sup>1</sup>, R. Allcock<sup>3</sup>, A. Heath<sup>1</sup>, P. B. Mitchell<sup>4,5</sup>, P. R. Schofield<sup>1,2</sup>, J. M. Fullerton<sup>1,2</sup>;

<sup>1</sup>Neuroscience Research Australia, Sydney, Australia, <sup>2</sup>School of Medical Sciences, University of New South Wales, Sydney, Australia, <sup>3</sup>Lotterywest State Biomedical Facility Genomics, Perth, Australia, <sup>4</sup>School of Psychiatry, University of New South Wales, Sydney, Australia, <sup>5</sup>Black Dog Institute, Prince of Wales Hospital, Sydney, Australia.

Introduction: Bipolar disorder (BD) is a heritable illness, contributed to by common variants of small effect and rare variants of higher penetrance. Pathogenic single nucleotide variants (SNV) of moderate effect are likely to be present in the encoded gene fraction (exome) and shared amongst individuals with BD in extended families.

Materials and methods: We selected 15 families (117 subjects), each containing 4 or more relatives with BD, to perform whole exome sequencing (WES) on the IonProton platform and copy number variant analysis (CNV) via CytoScanHD array (2 affected per family).

We selected SNVs and CNVs shared across multiple affected subjects, but also considered de novo variants. Linkage analysis using WES-derived genotypes refined family-specific linkage intervals. SNVs shared amongst affected and unaffected relatives were analysed combining all families for gene-set enrichment, gene ontology and KEGG pathways.

Results: The pool of SNVs shared amongst affected relatives was enriched in targets of the Fragile-X mental retardation 1 protein (FMRP) (P=8E-09) and post-synaptic density (PSD) genes (P=8E-03). The X-linked IRS4 gene carried a truncating mutation in 5 affected siblings in one family. This candidate gene displays restricted expression in the amygdala, and IRS4-/- female mice show compromised maternal behaviours. Our study also implicates the protocadherin genes, which act to mediate neuronal connectivity, with loss-of-function variants and deletions in several families.

Conclusions: Genetic approaches that combine WES, CNV and linkage analyses in extended families is effective in pinpointing genes and pathways that may contribute to the pathophysiology of the disorder.

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#### P09.035

#### Bipolar disorder genetic variation in the miR-708 gene and its binding targets

A. Fiorentino<sup>1,2</sup>, N. L. O'Brien<sup>2</sup>, D. Curtis<sup>3,4</sup>, A. McQuillin<sup>2</sup>;

<sup>1</sup>UCL Institute of Ophthalmology, London, United Kingdom, <sup>2</sup>UCL Molecular Psychiatry Laboratory, London, United Kingdom, <sup>3</sup>UCL Genetics Institute, London, United Kingdom,

<sup>4</sup>Centre for Psychiatry, Barts and the London School of Medicine and Dentistry, London, United Kingdom.

Introduction: rs12576775 has been found to be associated to bipolar disorder (BD) in GWAS. Downstream from the variant there is a genomic region with a high recombination rate that effectively restricts the size of associated region and implicates genes for miR-708, miR-5579 and the first exon of ODZ4. In this study the miR-708 gene, its surrounding region and its targets have been analysed for possible functional variants associated with BD. Methods: The surrounding areas of miR-708 were screened for variation using HRM analysis in 1,099 BD cases. Whole genome sequencing data from 99 BD subjects had been analysed for variation in potential miR-708 binding sites.

Results: A total of 3 variants were detected by HRM analysis across the region selected to be analysed. Only variants with a MAF lower than 0.01 in the general population were considered for genotyping in our case-control cohort. rs754333774 has been found in 3 bipolar cases, 2 schizophrenia (SCZ) cases and no controls. This variant is 260bp upstream miR-708 and could play a role in tuning the expression of the microRNA.

Four variants had been identified in miR-708 targets binding sites. None of

them had a markedly different allele frequencies in the BD compared to the general population.

Conclusion: We report a single recurrent BD and SCZ case only variant located in miR-708 gene that may have a role in the diseases susceptibility. These finding awaits replication in independent cohorts as does a functional analysis of the potential consequences of this variant. Grant references: MRC G1000708

## P09.036

### Exome sequencing of 81 individuals from 27 multiply affected families implicates the contribution of rare non-synonymous variants to bipolar disorder

**A. J. Forstner**<sup>1,2</sup>, S. B. Fischer<sup>3</sup>, L. M. Schenk<sup>1,2</sup>, J. Strohmaier<sup>4</sup>, A. Maaser<sup>1,2</sup>, J. Hecker<sup>5</sup>, C. Reinbold<sup>2</sup>, F. Degenhardt<sup>1,2</sup>, F. Streit<sup>6</sup>, H. Thiele<sup>6</sup>, P. Nürnberg<sup>6</sup>, J. Guzman Parra<sup>7</sup>, G. Orozco Diaz<sup>8</sup>, G. Auburger<sup>9</sup>, M. Albus<sup>10</sup>, M. Borrman-Hassenbach<sup>10</sup>, M. José González<sup>11</sup>, S. Gil Flores<sup>12</sup>, F. J. Cabaleiro Fabeiro<sup>13</sup>, F. del Río Noriega<sup>14</sup>, F. Perez Perez<sup>15</sup>, J. Haro González<sup>16</sup>, F. Rivas<sup>17</sup>, F. Mayoral<sup>17</sup>, S. Herms<sup>1,2,3</sup>, P. Hoffmann<sup>1,2,3</sup>, M. Rietschel<sup>6</sup>, M. M. Nöthen<sup>1,2</sup>, S. Cichon<sup>1,3,18</sup>,

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany, <sup>3</sup>Division of Medical Genetics and Department of Biomedicine, University of Basel, Basel, Switzerland,

<sup>4</sup>Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, University Medical Center Mannheim/University of Heidelberg, Mannheim, Germany, <sup>5</sup>Department of Genomic Mathematics, University of Bonn, Bonn, Germany,

<sup>6</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>7</sup>Mental Health Department, University Regional Hospital of Málaga, Institute of Biomedicine of Málaga, Málaga, Spain, <sup>8</sup>Unidad de Gestión Clínica del Dispositivo de Cuidados Críticos y Urgencias del Distrito Sanitario Málaga - Coin-Gudalhorce, Málaga, Spain, <sup>9</sup>Experimental Neurology, Department of Neurology, Goethe University Hospital, Frankfurt am Main, Germany, <sup>10</sup>Izar Amper Klinikum München Ost, kbo, Haar, Germany,

<sup>11</sup>University Regional Hospital of Málaga, Department of Mental Health, Instituto de Biomedicina de Málaga (IBIMA), Málaga, Spain, <sup>12</sup>Hospital of Jaen, Department of Mental Health, Jaén, Spain, <sup>13</sup>Hospital of Jerez de la Frontera, Department of Mental Health, Jerez de la Frontera, Spain, <sup>14</sup>Hospital of Puerto Real, Department of Mental Health, Cádiz, Spain, <sup>15</sup>Hospital Punta de Europa, Department of Mental Health, Algeciras, Spain, <sup>16</sup>Department of Psychiatry, Hospital Regional Universitario Carlos Haya Málaga, Málaga, Spain, <sup>17</sup>Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany.

Bipolar disorder (BD) is a severe psychiatric disorder affecting about 1% of the world's population. The highly heritable disease is characterized by recurrent episodes of mania and depression.

As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD, rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In the present study we investigated 226 individuals of 70 large multiplex BD families of German and Spanish origin by whole exome sequencing (Illumina HiSeq2500 platform). For data analysis the Varbank pipeline of the Cologne Center for Genomics was used. We filtered for rare (minor allele frequency <0.1%) and non-synonymous variants that are shared within each family and are predicted to be damaging by at least four of five different bioinformatics tools.

So far, we identified 955 rare, segregating and potentially damaging variants in 889 different genes. Pathway analysis of 269 genes with a Residual Variation Intolerance Score <25% showed a significant enrichment ( $p<0.001$ ) for 37 pathways including neuron differentiation and axon development. In addition, 58 genes were implicated by rare variants in at least two unrelated families. These comprise NRXN2 which encodes a synaptic cell-adhesion molecule connecting pre- and postsynaptic neurons and mediating synaptic signaling.

Our preliminary results suggest that rare and highly-penetrant variants in genes involved in synaptic signaling and neuron development contribute to BD. Further investigation of the remaining families and follow up analyses are currently underway and will be presented at the upcoming conference.

Bipolar disorder (BD) is a highly heritable mood disorder with a lifetime prevalence of about 1%. Models of illness are most consistent with a polygenic contribution of common and rare variants to disease susceptibility. As the cumulative impact of common alleles may only explain around 38% of the phenotypic variance, rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In the present study we investigated the role of rare variants in BD by conducting whole-exome sequencing of 81 individuals from 27 large multiply affected Spanish and German families. In each family 3 genetically distant affected individuals were selected for exome sequencing. For variant calling and data analysis, the VARBANK pipeline of the Cologne Center for Genomics was used. We focused on rare non-synonymous variants (minor allele frequency <0.1%) that were shared among all three affected individuals and predicted to be potentially/probably damaging by at least 3 of 5 applied bioinformatics tools. Segregating variants in genes affected in at least two independent families were validated using Sanger sequencing.

Among all families we identified 404 rare non-synonymous and potentially damaging variants spanning 393 genes. 8 genes harbored rare segregating variants in at least two independent families including RGS12 which is known to play an important role in promoting and maintaining neuronal differentiation. Pathway analysis of all genes with a Residual Variation Intolerance Score <10% revealed significant enrichment for 18 pathways after correction for multiple testing including synaptic membrane and axon guidance.

A.J.Forstner&S.B.Fischer contributed equally to this work.

## P09.037

### Exome sequencing of European families densely affected with bipolar disorder reveals rare variants in synaptic genes contributing to disease etiology

**A. Maaser**<sup>1,2</sup>, J. Strohmaier<sup>2</sup>, K. U. Ludwig<sup>1,2</sup>, F. Degenhardt<sup>1,2</sup>, F. Streit<sup>2</sup>, L. M. Schenk<sup>1,2</sup>, A. C. Koller<sup>1,2</sup>, S. B. Fischer<sup>4</sup>, H. Thiele<sup>5</sup>, P. Nürnberg<sup>6</sup>, J. Guzman Parra<sup>7</sup>, G. Orozco Diaz<sup>8</sup>, G. Auburger<sup>9</sup>, M. Albus<sup>10</sup>, M. Borrman-Hassenbach<sup>9</sup>, M. José González<sup>10</sup>, S. Gil Flores<sup>11</sup>, F. J. Cabaleiro Fabeiro<sup>12</sup>, F. del Río Noriega<sup>13</sup>, F. Perez Perez<sup>14</sup>, J. Haro González<sup>15</sup>, F. Rivas<sup>16</sup>, F. Mayoral<sup>17</sup>, S. Herms<sup>1,2,4</sup>, P. Hoffmann<sup>1,2,4</sup>, S. Cichon<sup>1,4,17</sup>, M. Rietschel<sup>6</sup>, M. M. Nöthen<sup>1,2</sup>, A. J. Forstner<sup>1,2</sup>,

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany, <sup>3</sup>Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, University Medical Center Mannheim/University of Heidelberg, Mannheim, Germany, <sup>4</sup>Division

of Medical Genetics and Department of Biomedicine, University of Basel, Basel, Switzerland, <sup>5</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>6</sup>Mental Health Department, University Regional Hospital of Málaga, Institute of Biomedicine of Málaga, Málaga, Spain, <sup>7</sup>Unidad de Gestión Clínica del Dispositivo de Cuidados Críticos y Urgencias del Distrito Sanitario Málaga - Coin-Gudalhorce, Málaga, Spain, <sup>8</sup>Experimental Neurology, Department of Neurology, Goethe University Hospital, Frankfurt am Main, Germany, <sup>9</sup>Izar Amper Klinikum München Ost, kbo, Haar, Germany, <sup>10</sup>University Regional Hospital of Málaga, Department of Mental Health, Instituto de Biomedicina de Málaga (IBIMA), Málaga, Spain, <sup>11</sup>University Hospital Reina Sofia, Department of Mental Health, Córdoba, Spain, <sup>12</sup>Hospital of Jaen, Department of Mental Health, Jerez de la Frontera, Spain, <sup>13</sup>Hospital of Jerez de la Frontera, Department of Mental Health, Jerez de la Frontera, Spain, <sup>14</sup>Hospital of Puerto Real, Department of Mental Health, Cádiz, Spain, <sup>15</sup>Hospital Punta de Europa, Department of Mental Health, Algeciras, Spain, <sup>16</sup>Department of Psychiatry, Hospital Regional Universitario Carlos Haya Málaga, Málaga, Spain, <sup>17</sup>Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany.

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Our preliminary results suggest that rare and highly-penetrant variants in genes involved in synaptic signaling and neuron development contribute to BD. Further investigation of the remaining families and follow up analyses are currently underway and will be presented at the upcoming conference.

## P09.038

### CADASIL: Variants of unknown significance (VUS) may affect mRNA splicing of the NOTCH3 gene

**H. Vlaskova**<sup>1</sup>, M. Hruba<sup>2</sup>, H. Treslova<sup>1</sup>, M. Hnizdova Bouckova<sup>1</sup>, L. Dvorakova<sup>1</sup>, R. Matej<sup>3</sup>, J. Sikora<sup>1</sup>, H. Hulkova<sup>1</sup>, V. Kozich<sup>1</sup>,

<sup>1</sup>Institute of Inherited Metabolic Disorders, University Hospital and 1st Faculty of Medicine, Charles University, Prague 2, Czech Republic, <sup>2</sup>Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic,

<sup>3</sup>Department of Pathology and Molecular Medicine, Thomayer Hospital, Prague 4, Czech Republic.

Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterized by migraine, recurrent stroke, and dementia. CADASIL is caused by mutations in the NOTCH3 gene. Typical NOTCH3 mutations result in the addition or the loss of a cysteine residue. Nonetheless, other types of NOTCH3 mutations were recently identified to also associate with CADASIL.

Patients and Methods: In a cohort of 220 patients suspected of having CADASIL, we detected by Sanger sequencing typical mutations in 70 individuals with 24 different alleles. In addition, we observed 7 variants of unknown significance (VUS) in the other 11 patients (p.G73V, p.R374=, p.S497L, p.T575=, p.R607H, p.V644D, c.2410+97A>T). In this study we examined the effects of 3 of these VUSs on mRNA splicing by analysing cDNA. Nucleic acids were isolated from peripheral blood leukocytes.

Results: The variants p.G73V and p.S497L did not exhibit any effect on structure alteration or mRNA stability. However, in-silico analyses predicted that a novel variant (c.1725G>A, p.Thr575=) generates a new donor splice site in exon 11 with subsequent missplicing. Indeed, the sequencing analyses revealed a deletion of 120 nucleotides (r.1607\_1726del120) that putatively causes an inframe deletion of 40 amino acids including 6 cysteine residues. This abnormality is expected to lead to uneven disulfide pairing and consequently to abnormal folding of the Notch3 protein.

Conclusions: Our study demonstrates that non-typical NOTCH3 variants such as synonymous or missense mutations not affecting directly cysteine residues may be a potential molecular cause of CADASIL.

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**P09.040****Copy number variations in psychiatric patients with intellectual disability and catatonia: an exploratory study***A. M. L. Vogels;**Centre for Human Genetics, Leuven, Belgium.*

**Introduction:** Catatonia is a motor dysregulation syndrome co-occurring with a variety of psychiatric and somatic disorders. The good response to treatment with benzodiazepines and electroconvulsive therapy indicates a neurobiological background. Environmental factors as well as genetic factors play a role in the etiopathogenesis. Research on the genetic aetiology is limited. We hypothesize that copy number variations known to be risks factors for neurodevelopmental disorders may play a role in the aetiology of catatonia. The aim of this study is to describe the CNVs in a population of psychiatric patients with an intellectual disability and catatonia. **Methods:** Fifteen intellectually disabled adults admitted to a psychiatric inpatient unit and diagnosed with catatonia were selected for genetic examination. Medical files were analysed retrospectively to collect data on cognitive functioning and psychiatric diagnosis. A clinical genetic examination was performed. Blood samples were taken for molecular karyotyping (Comparative Genomic Hybridisation). **Results:** CNVs, including 5 duplications and 3 deletions, were detected in 8 of the 15 patients (53%). In 2 of these patients a microdeletion including SHANK3 was found. Psychiatric diagnoses in these patients are autism, psychotic and affective disorders. Intellectual disability ranged from borderline to severe disability. **Conclusion:** CNVs occurred in half of intellectually disabled psychiatric adults with catatonia. These findings suggest that SHANK3del may play a role in the aetiology of catatonia in intellectually disabled patients. Cognitive functioning ranged from borderline to severe intellectual disability. Genetic research in children as well as in adults with intellectual disabilities and psychiatric comorbidity is important and meaningful.

*University of Washington, Seattle, WA, United States, <sup>5</sup>Department of Pathology, University of Washington, Seattle, WA, United States.*

**Introduction:** Cerebellar dysfunction has a large impact on both cognitive and motor behavior and mounting evidence implicates the cerebellum in autism spectrum disorders (ASD). However, the impact of ASD genes on cerebellar development and function is largely unknown. Here, we defined the 15 post-conception week (pcw) human fetal cerebellar transcriptome and hypothesized that ASD genes have neuron-type specific expression.

**Materials and Methods:** Laser capture microdissection (LCM) of intact cerebella from two 15 pcw fetuses enabled isolation of Purkinje cell (PC) and granule cell (GC) progenitor neurons. High-quality RNA was isolated ( $RIN > 8$ ), sequencing libraries using TruSeq RNA Access kit were constructed, barcoded, and sequenced using the Illumina HiSeq 2000. Paired end reads were aligned to the human genome HG19 using Tophat2, genes and counts were summarized using HTSeq. Gene-level differential expression was analyzed using DESeq2.

**Results:** On average, RNA-seq generated 18 million high-quality reads per sample, 96% of which mapped uniquely. Hierarchical clustering using the most variable genes within the dataset distinguished LCM-isolated PC and GC samples, including several known PC- or GC-specific genes. ASD genes generated from exome sequencing studies were expressed either throughout the cerebellum or restricted to PC neurons.

**Conclusions:** LCM is an effective method to enrich for developing PC and GC neurons in human fetal cerebellum. ASD genes are expressed throughout mid-gestation cerebellum or restricted to PC neurons, further substantiating cerebellar neuroimaging and neuropathological findings in ASD, and providing a potential neuronal therapeutic target.

**Grant references**

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**P09.041****Our experience in Genetic testing of Cerebrous Cavernous Malformations: Revisiting indication criteria**

*M. Borregán, A. Esteve, A. Cueto, T. Vendrell, I. Valenzuela, J. Vilalta, A. Outeríal, A. Tenes, E. Tizzano, O. Diez;*  
*Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron, Barcelona, Spain, Barcelona, Spain.*

**INTRODUCTION:** Cerebral cavernous malformations (CCM) are vascular abnormalities that occur mostly sporadically and associated with a single lesion. CCM can also be inherited in an autosomal dominant pattern characterized by the presence of multiple lesions, but much less frequently. Three known genes KRIT1, MGC4607 and PDCD10 are usually studied when multiple cavernomas are found. When a single abnormality is found the yield of the molecular study is very low, but little is known when lesions are described as suggestive and/or not clearly quantified as multiple but as discrete. We aimed to analyse our casuistic to update recommendations for testing and counselling of families.

**MATERIALS AND METHODS:** We conducted a detailed clinical molecular analysis (sequencing and MLPA of KRIT1, MGC4607 and PDCD10) in 63 CCM patients referred from the Neurosurgery Department of our Hospital, in an effort to analyze the correlation of clinical and RMN findings with the molecular results.

**RESULTS:** We identified mutations in 35% of cases, which was increased up to 75% when only multiple lesions (n=28) were considered. No mutation was revealed in patients with a single cavernoma (n=9) neither in those with 2 lesions (n=18). Only one from 8 patients with more than two and up to four discrete number of lesions showed a mutation correlating with a positive family history.

**CONCLUSIONS:** Patients with a discrete number of malformations should be settle apart from the recognized familial multiple CCM to optimize GT yield and establish clear recommendations for clinical management and assessment of these families.

**P09.043****Expert recommendations for the laboratory diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): diagnostic algorithm and best practice guidelines for a timely diagnosis**

*E. Izzo<sup>1</sup>, M. Alsayed<sup>2</sup>, D. Burke<sup>3</sup>, J. Cohen-Pfeffer<sup>4</sup>, J. D. Cooper<sup>5</sup>, L. Dvořáková<sup>6</sup>, M. Fietz<sup>7</sup>, R. Giugliani<sup>8</sup>, H. Jahnová<sup>5</sup>, Z. Lukacs<sup>9</sup>, S. Mole<sup>9</sup>, I. Noher de Halac<sup>10</sup>, D. Pearce<sup>11</sup>, A. Schulz<sup>12</sup>, N. Specchio<sup>13</sup>, W. Xin<sup>14</sup>, N. Miller<sup>1</sup>,*

<sup>1</sup>*BioMarin Pharmaceutical Inc, Novato, CA, United States, <sup>2</sup>Department of Medical Genetics, Alfaisal University and King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, <sup>3</sup>Chemical Pathology, Camelia Botnar Laboratories, Great Ormond Street Hospital, London, United Kingdom, <sup>4</sup>Institute of Psychiatry, Psychology, & Neuroscience, King's College London, London, United Kingdom, <sup>5</sup>Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic, <sup>6</sup>Department of Diagnostic Genomics, PathWest Laboratory Medicine WA, Nedlands, Australia, <sup>7</sup>Serviço de Genética Médica HCPA, Departamento de Genética UFRGS, Porto Alegre, Brazil, <sup>8</sup>Newborn screening and Metabolic Diagnostics unit, Hamburg University Medical Center, Hamburg, Germany, <sup>9</sup>Molecular Cell Biology, MRC Laboratory for Molecular Cell Biology, University College London, London, United Kingdom, <sup>10</sup>Universidad Nacional de Córdoba, Facultad de Ciencias Médicas, Córdoba, Argentina, <sup>11</sup>Sanford Children's Health Research Center, Sioux Falls, SD, United States, <sup>12</sup>Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, <sup>13</sup>Department of Neuroscience, Bambino Gesù Children's Hospital, Rome, Italy, <sup>14</sup>Neurogenetics DNA Diagnostic Lab, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States.*

Neuronal ceroid lipofuscinoses (NCLs), a heterogeneous group of lysosomal storage disorders, include the rare autosomal recessive neurodegenerative disorder CLN2 disease (CLN2). CLN2 is due to mutations in *TPP1/CLN2* gene causing tripeptidyl-peptidase-1 (TPP1) enzyme deficiency. Classic late-infantile CLN2 has pediatric onset with initial symptoms of seizures and language delay followed by progressive dementia, motor and visual deterioration and early death. Variant phenotypes occur more rarely. CLN2 diagnosis is based on laboratory testing following clinical suspicion. Early diagnosis is key to optimizing clinical care and future therapies outcomes, yet delays in diagnosis are common due to low disease awareness, non-specific initial symptoms and limited diagnostic testing access in some regions.

In May 2015, international experts met to recommend best laboratory practices for early CLN2 diagnosis. When clinical signs suggest NCLs, TPP1 activity should be the first test performed (along with palmitoyl-protein-thioesterase-1 to exclude CLN1). However, since reaching initial suspicion of CLN2 and NCLs is challenging, where available, use of epilepsy gene panels to investigate unexplained seizures in childhood is endorsed. These panels should include *TPP1/CLN2* besides genes for other NCLs lacking biochemical tests.

Diagnostic TPP1 enzyme test in leukocytes is well established and robust and in DBS is considered diagnostic if followed by molecular testing. Future methods to measure TPP1 activity via MS/MS may improve DBS-based

**P09.042****Autism-related gene expression is spatially restricted in mid-gestation human fetal cerebellum**

*I. A. Glass<sup>1</sup>, K. Aldinger<sup>2</sup>, A. Timms<sup>3</sup>, M. Deng<sup>4</sup>, T. Naluai-Cecchini<sup>4</sup>, D. O'Day<sup>4</sup>, M. Weaver<sup>5</sup>, M. O. Dorschner<sup>6</sup>, W. B. Dobyns<sup>1</sup>, K. J. Millen<sup>1</sup>,*

<sup>1</sup>*Department of Pediatrics, University of Washington; Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, United States, <sup>2</sup>Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, Seattle, WA, United States, <sup>3</sup>Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA, United States, <sup>4</sup>Department of Pediatrics,*

TPP1 testing sensitivity allowing also future newborn screening. To confirm clinical suspicion of CLN2, the recommended gold standard for laboratory diagnosis is demonstrating deficient TPP1 activity and/or detecting causative mutations in each allele of *TPP1/CLN2* gene.

#### P09.044

#### Novel frameshift mutation in *CHD8* causes familial autism spectrum disorder with intrafamilial clinical variability

**T. Reimand<sup>1</sup>, K. Noormets<sup>2</sup>, I. Talvik<sup>3</sup>, S. Pajusalu<sup>4</sup>;**

<sup>1</sup>Department of Genetics, Tartu University Hospital, Tartu, Estonia 2. Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia 3. Department of Pediatrics, Institute of Clinical Medicine, Tartu, Tartu, Estonia, <sup>2</sup>Department of Neurology, Children's Clinic of Tartu University Hospital, Tartu, Tartu, Estonia, <sup>3</sup>Tallinn Children's Hospital, Tallinn, Estonia, <sup>4</sup>Department of Genetics, Tartu University Hospital, Tartu, Estonia 2. Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, Tartu, Estonia.

Recently disruptive mutations in chromodomain helicase DNA-binding domain 8 (*CHD8*) gene were shown to cause neurocognitive syndrome involving autism spectrum disorder (ASD) and macrocephaly. We describe a familial case of *CHD8*-related syndrome showing variable clinical phenotype among three affected family members.

The proband was a girl, first referred to clinical geneticist at the age of 2y5m due to global developmental delay and facial dysmorphism. Her occipito-frontal circumference (OFC) was +2 SD. At the age of 6y she had mild intellectual disability, ASD, and epilepsy. Her communication skills improved with initiation of antiepileptic treatment. Novel heterozygous frameshift mutation in *CHD8* was detected by next generation sequencing panel (4800 genes): NM\_001170629.1:c.2423\_2424del, p.(Arg808Lysfs\*12).

Her brother had congenital heart defect, bilateral inguinal hernias, and facial dysmorphism. He started to walk at the age of 2y. At 3y10m his OFC was +1.75 SD. He had no speech, and the developmental delay and ASD phenotype were more severe than in his sister. Their father had inguinal hernia, problems with making friends during childhood, and some learning difficulties. At the age of 29y he has facial dysmorphism, and poor eye contact but no remarkable communication problems. His OFC is 61 cm (> +2 SD). The same *CHD8* mutation was confirmed in both brother and father.

In conclusion, our study suggests that the clinical picture caused by *CHD8* mutations can vary significantly between family members. The most prevalent feature is ASD, but macrocephaly is not present in every individual.

#### P09.045

#### Case report: exome sequencing of a family with childhood disintegrative disorder.

**F. Ceroni<sup>1</sup>, M. Absoud<sup>2</sup>, G. Baird<sup>2</sup>, A. Velayos-Baeza<sup>1</sup>, D. Newbury<sup>1</sup>;**

<sup>1</sup>Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom, <sup>2</sup>Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom.

Childhood disintegrative disorder (CDD) is a very rare but devastating neurodevelopmental condition characterised by rapid and severe decline in developmental skills (communication, play, self-care, cognition) in children with apparently normal previous development for the first 2 years of life. The regression results in significant long-term impairments in social communication skills, similar to features of Autism Spectrum Disorder (ASD) with severe intellectual disability. Extensive neurometabolic investigations usually do not reveal an underlying aetiology. We report an exome sequencing study of a family with two affected children and neurotypical parents. Pathway analyses of rare and potentially damaging variants highlighted two variants in genes involved in intracellular trafficking and recruitment of proteins to the centrosome: both children carry a maternal stop codon in PCM1 (NP\_006188, p.E1912X) and a paternal nonsynonymous change in ALMS1 (NP\_055935, p.S763N). PCM1 is an essential component of the centriolar satellites and interacts with several proteins, including DISC1 (disrupted in schizophrenia 1) and BBS4 (Bardet-Biedl Syndrome 4). Mutations in ALMS1 can cause Alström Syndrome, a rare recessive multi-system cilopathy, closely related to the Bardet-Biedl Syndrome (BBS). The biological functions of ALMS1 are still being elucidated, but roles in cilium function and maintenance, intracellular trafficking, signaling pathways and cell cycle regulation have been suggested. Although the functional interaction between ALMS1 and PCM1 needs to be further investigated, the participation of the two proteins to the same cellular network, which has been previously implicated in other neurodevelopmental disorders, led us to hypothesize a possible compound effect of the two identified variants.

#### P09.046

#### Structural chromosome 21-specific instability in the diseased brain

**Y. B. Yurov<sup>1,2,3</sup>, S. G. Vorsanova<sup>2,1,3</sup>, T. Liehr<sup>4</sup>, A. D. Kolotii<sup>2,1</sup>, I. Y. Iourov<sup>1,2,5</sup>;**

<sup>1</sup>National Research Center of Mental Health, Moscow, Russian Federation, <sup>2</sup>Research and Clinical Institute for Pediatrics named after YE Veltishev at the Pirogov Russian National Research Medical University, Ministry of Health, Moscow, Russian Federation, <sup>3</sup>Moscow State University of Psychology and Education, Moscow, Russian Federation, <sup>4</sup>Institute of Human Genetics, Jena, Germany, <sup>5</sup>Department of Medical Genetics, Russian Medical Academy of Postgraduate Education, Ministry of Health, Moscow, Russian Federation.

Introduction. Chromosome 21 aneuploidy has been shown to affect the diseased brain especially in individuals with Alzheimer's disease (AD). However, structural chromosome 21-specific instability has never been a target for molecular (neuro)cytogenetic studies of the diseased brain. Here, we have addressed chromosome 21 structural variations in single cells of the AD, schizophrenia and autism brain as well as in the unaffected brain.

Materials and Methods. Ten AD, 18 schizophrenia, 12 autism and 20 age- and sex matched samples of the postmortem brain (frontal cortex, Brodmann area 10) were studied by interphase chromosome-specific multicolor banding (ICS-MCB) and NeuN- immunohistochemistry.

Results. We were able to show that rare recurrent rearrangements of chromosome 21 or structural chromosome 21-specific instabilities are found almost exclusively in the AD brain. These were isochromosomes 21q, 21q22->qter losses and chromosome 21 breaks at 21q21 and 21q22 affecting 0.6-3.7% of cells. NeuN immunohistochemistry has indicated that the instability randomly affects NeuN-positive and NeuN-negative cell nuclei in contrast to chromosome 21 breaks, which were more prevalent in NeuN-positive cell nuclei.

Conclusions. Our data suggests that chromosome 21-specific instability is able to contribute to the AD pathogenesis. One can hypothesize chromosome 21 breaks confined to NeuN-positive cells of the AD brain to result from the dysregulation of DNA replication/reparation or DNA damage response pathways. Supported by the Russian Science Foundation (Grant #14-35-00060) and ERA.Net RUS Plus Programme.

#### P09.047

#### Population genetics of SNPs, associated with cognitive traits and Alzheimer's diseases, in populations of Russia

**M. Swarovskaya, 634050<sup>1</sup>, V. Stepanov<sup>1,2</sup>, A. Bocharova<sup>1</sup>, A. Marusin<sup>1</sup>, K. Vagaitseva<sup>1,2</sup>;**

<sup>1</sup>Institute for Medical Genetics, Tomsk, Russian Federation, <sup>2</sup>Tomsk State University, Tomsk, Russian Federation.

Introduction. Cognitive functions in normal population and in various forms of dementia, the main of which is Alzheimer's disease (AD), demonstrate a high level of heritability as well as considerable individual variability. Population background appears to be an important factor in personalized health care and predictive medicine directed to prevention of common diseases, including AD. The aim of this study was to estimate the variability of genetic markers strongly associated with cognitive performance (CP) and AD across the multiple Eurasian populations.

Methods. We selected 53 single nucleotide polymorphisms (SNPs) reported in recent GWAS as highly significantly associated with AD and CP, and investigated the population variability of these markers in 20 populations of Russia representing Eastern Europe, Caucasus, Central Asia, North Asia and Siberia.

Results. Considerable between-population variability in allele frequencies was found. Average genetic diversity ranged from 0.34 in Belorussians to 0.39 in Kazakhs. Population differentiation measured by Fst varied widely across the loci studied (from 0.005 to 0.144). Mean Fst for all SNPs was 0.048. Mean Fst for AD (0.033) proved to be substantially lower than population differentiation for CP (0.053). Allele frequencies tend to correlate between populations according their geographical locations.

Conclusion. Our data indicate wide inter-population variation in the frequency of alleles associated with cognitive functions in norm and pathology. This variability may contribute to variation in genetic predisposition to dementia in ethnically different populations and to population-specific patterns of associations.

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**P09.048****Moroccan patient with CCDC88C related congenital hydrocephalus: A case report**W. Smaili<sup>1,2</sup>, I. C. Jaouad<sup>1,2</sup>, A. Baumer<sup>3</sup>, A. Rauch<sup>3</sup>, A. Sefiani<sup>1,2</sup>;<sup>1</sup>Centre de Génétique Humaine – Faculté de Médecine et de Pharmacie- Université Mohamed V, Rabat, Morocco, <sup>2</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, <sup>3</sup>Institute for Medical Genetics, University of Zurich, Zurich, Switzerland.

Nonsyndromic congenital hydrocephalus is a rare condition characterized by abnormal accumulation of cerebrospinal fluid that leads to in utero onset of ventricles enlargement.

The disease etiology is poorly clarified and the most frequent monogenic form of congenital hydrocephalus is due to hemizygous mutations in the *L1CAM* gene. This form is X-linked and clinically characterised by a combination of hydrocephalus in association with adducted thumbs. Besides *L1CAM*, autozygosity mapping revealed that *CCDC88C* mutations seem to cause a distinct non-syndromic complex hydrocephalus inherited in autosomal recessive way, with apparently normal psychomotor development despite the impressive prenatal cerebral phenotype. This gene was identified as a further essential component of the Wnt signalling pathway in human brain development.

We hereby report the case of a Moroccan girl, born of consanguineous parents, with congenital nonsyndromic hydrocephalus. Fetal ultrasound at 27 weeks' gestation showed enlarged ventricles. Brain MRI at birth showed triventricular asymmetric hydrocephalus with deviated appearance of the left lateral ventricle. The child had seizures with mild psychomotor delay. *CCDC88C* gene sequencing found a homozygous mutation in the exon 30: c.5265\_5266delCA (p.Phe1755Leufs\*4), parents were both heterozygous for this mutation.

Identification of *CCDC88C* autosomal recessive congenital hydrocephalus allowed us to offer adequate support to the patient and to provide genetic counseling to parents.

**P09.049****Severe neurodegenerative phenotype associated with progressive loss of myelination caused by a homozygous nonsense mutation in CSTB**A. O'Brien<sup>1</sup>, C. R. Marshall<sup>2,3</sup>, S. Blaser<sup>4</sup>, P. Ray<sup>2,5</sup>, G. Yoon<sup>1,6</sup>,<sup>1</sup>Division of clinical and metabolic genetics, department of paediatrics, Hospital for Sick Children, Toronto, ON, Canada, <sup>2</sup>Department of paediatric laboratory medicine, Hospital for Sick Children, Toronto, ON, Canada, <sup>3</sup>The centre for applied genomics, Hospital for Sick Children, Toronto, ON, Canada, <sup>4</sup>Division of paediatric neuroradiology, Hospital for Sick Children, Toronto, ON, Canada, <sup>5</sup>Department of molecular genetics, University of Toronto, Toronto, ON, Canada, <sup>6</sup>Division of neurology, Department of paediatrics, Hospital for Sick Children, Toronto, ON, Canada.

Introduction: Mutations of the cystatin B gene (CSTB; OMIM 601145) are known to cause Unverricht-Lundborg disease, or progressive myoclonic epilepsy-1A (EPM1A, MIM #254800). Most patients are homozygous for an expanded (>30) dodecamer repeat in the promoter region of CSTB. Some patients are compound heterozygotes for the dodecamer repeat and a point mutation, and these patients generally have an earlier age of onset and more severe phenotype. Case Report: Two sisters born to consanguineous parents of Sri-Lankan descent presented with profound global developmental delay, microcephaly, cortical blindness, and central hypotonia with peripheral hypertonia. Neither sibling ever developed head control, independent sitting or ambulation, and never developed speech. The elder sister had a seizure disorder while the younger one had multiple electroencephalograms which failed to detect seizures. Clinical examination of both sisters revealed profound microcephaly, beaked nose with overhanging columella, supernumerary teeth, and short neck. On serial brain imaging, they had progressive atrophy of the corpus callosum, and diffuse hypomyelination with progressive loss of myelination. Results: Exome sequencing revealed both siblings to be homozygous for a c.218dup (p.His75Serfs\*) mutation in exon 3 of CSTB. Their mother was a confirmed carrier of the mutation; the father was unavailable for testing. Conclusion: The neuroimaging features of our patients are consistent with those observed in Cstb-knockout mice, which supports the hypothesis that disease severity is inversely correlated with the amount of residual functional cystatin B protein. To our knowledge, this is the first report of a homozygous nonsense mutation in CSTB in humans.

**P09.050****Further case of the rare congenital neuronal ceroid lipofuscinosis form confirmed by targeted exome sequencing showing a novel cathepsin D mutation**K. Varvagiannis<sup>1</sup>, J. Fluss<sup>1</sup>, A. Poretti<sup>2</sup>, M. H. Billieux<sup>1</sup>, R. De Luca<sup>1</sup>, P. Rimensberger<sup>1</sup>, S. Hanquinet<sup>1</sup>, M. Guipponi<sup>1</sup>, E. Hammar<sup>1</sup>, P. Makrythanasis<sup>1,3</sup>, M. Lidgren<sup>1,3</sup>, J. L. Blouin<sup>1</sup>, R.Steinfeld<sup>4</sup>, I. Kern<sup>1</sup>, S. E. Antonarakis<sup>1,3</sup>, S. Fokstuen<sup>1</sup>,<sup>1</sup>University Hospitals of Geneva, Geneva, Switzerland, <sup>2</sup>The Johns Hopkins School of Medicine, Baltimore, MD, United States, <sup>3</sup>University of Geneva, Geneva, Switzerland,<sup>4</sup>University of Göttingen, Göttingen, Germany.

Neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative lysosomal storage disorders. Neuronal ceroid lipofuscinosis-10 (CLN10 – OMIM #610127) due to cathepsin D deficiency is the most severe form, characterized by prenatal onset, and presents at birth with congenital microcephaly, extensive neuronal loss, neonatal seizures and early death. Few cases have been reported to date.

We report on a female baby, born at 37 4/7 gestational weeks to consanguineous parents after an uneventful pregnancy. Apgar score was 2/2/3. At birth the neonate did not have sufficient spontaneous breathing and needed intubation. In addition, she developed neonatal seizures. Brain MRI revealed severe and diffuse atrophy on both the brain and the cerebellum. The signal on the thalamus was decreased on T2 and increased on T1. The spectroscopy showed an important deficit of NAA and elevated lactate. Support was withdrawn 5 days after birth, given the poor prognosis. Based on the neuroimaging findings, a neurodegenerative disease with prenatal onset, like CLN10, was suspected.

We performed exome sequencing with targeted bioinformatic analysis of 225 genes responsible of prenatal/perinatal encephalopathy including the cathepsin D gene (*CTSD*). The results revealed homozygosity for a novel 3 base-pair deletion in *CTSD* presumed to lead to the deletion of a phenylalanine residue. Both parents were heterozygous carriers of the variant (NM\_001909.4:c.686\_688del). Functional studies were performed and demonstrated absence of cathepsin D enzyme activity as well as undetectable protein product in Western Blot, thus further suggesting pathogenicity of this variant. These results confirmed the diagnosis of CLN10.

**P09.051****Mutations of the mTORC1-regulating complex GATOR1 in focal epilepsies**S. Baldassari<sup>1</sup>, L. Licchetta<sup>2</sup>, C. Marconi<sup>1</sup>, C. T. Myers<sup>3</sup>, F. Palombo<sup>1</sup>, P. Magini<sup>1</sup>, H. C. Mefford<sup>3</sup>, M. Seri<sup>1</sup>, LICE NFLE Study Group, P. Tinuper<sup>2</sup>, F. Bisulli<sup>2</sup>, T. Pippucci<sup>1</sup>,<sup>1</sup>Medical Genetics Unit, Dept of Medical and Surgical Sciences, University of Bologna - Sant'Orsola-Malpighi Hospital, Bologna, Italy, <sup>2</sup>IRCCS Istituto delle Scienze Neurologiche di Bologna - Dept of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, <sup>3</sup>Dept of Pediatrics, University of Washington, Seattle, WA, United States.

Focal epilepsies (FEs) account for nearly 60% of all epileptic syndromes and are characterized by epileptic discharges originating from a limited area of the brain with heterogeneous etiologies. The most genetically characterized forms of FEs are Nocturnal Frontal Lobe Epilepsy (NFLE), Epilepsy with Auditory Features (EAF) and Familial Focal Epilepsy with Variable Foci (FFEVF). The genes associated with these conditions cumulatively explain a low percentage of the cases. We performed Whole Exome Sequencing (WES) analysis on a cohort of patients with FE, including 60 NFLE probands and 25 EAF probands, together with their healthy parents in the case of sporadic patients (trios) or affected relatives when available. The genetic analysis revealed 7 variants in the known gene DEPDC5 and 1 NPRL2 variant, which encode two components of the GATOR1 complex, together with NPRL3, negatively regulating mTORC1 signaling cascade. Our findings contributed to the discovery of the involvement of all the GATOR1 complex genes in the pathogenesis of up to 9% of FEs, in contrast to the concept that specific seizure semiologies point to the main involvement of specific brain areas. We therefore screened for mutations in GATOR1 encoding genes in an additional cohort of 65 patients with FEs using Multiple Inversion Probe technology, finding a novel nonsense variant in NPRL3 in one additional patient. The deregulation of GATOR1 in FEs have important implications for patients' diagnosis and treatment, suggesting GATOR1/mTOR signaling as a potential key target for effective anti-epileptic drugs.

**P09.052****DHCR7 mutations causing Smith-Lemli-Opitz syndrome in a patient cohort from Balkan region**A. Kirov<sup>1,2</sup>, T. Todorov<sup>2</sup>, V. Plaicasu<sup>3</sup>, K. Kovacheva<sup>4</sup>, A. Todorova<sup>1,2</sup>,<sup>1</sup>Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria, <sup>3</sup>Mother and Child's Care Institute IOMC "Prof.dr. Alfred Rusescu", Bucharest, Romania, <sup>4</sup>UMBAL "Dr. G. Stranski", Pleven, Bulgaria.

Smith-Lemli-Opitz syndrome (SLOS) is a rare metabolic disorder inherited in an autosomal recessive pattern. Clinically it is characterized by developmental abnormalities, such as psychomotor retardation, craniofacial anomalies, limb malformations, pre- and postnatal failure to thrive, genital particularities and variable structural anomalies of internal organs. From biochemical point of view this condition is characterized by low plasma cho-

lesterol levels and high concentrations of the cholesterol precursor 7-dehydrocholesterol (7DHC) due to the deficient activity of 7DHC  $\Delta 7$ -reductase encoded by the DHCR7 gene.

Here we report 8 unrelated patients from Romanian and Bulgarian origin with different mutations in the DHCR7 gene. We identified the following mutations: c.278C>T; p.(Thr93Met) - allele frequency 18,75%; c.293A>C, p.(Gln98Pro) - 6,25%; c.355delC, p.(Val126\*) - 6,25%; c.452G>A; p.(Trp151\*) - 37,5%; c.964-1G>C - 6,25%; c.976G>T; p.(Val326Leu) - 12,50%; c.1295A>G; p.(Tyr432Cys) - 6,25%; c.1315delC; p.(Leu439Cysfs\*2) - 6,25%. The mutations were detected by Sanger sequencing (using BigDye terminator v3.1 Cycle Sequencing Kit). The DNA sequencing results were compared to NCBI Reference Sequence: NM\_001360.2. The genetically confirmed diagnosis allowed genetic counseling and prenatal diagnosis in the affected families.

Generally the incidence of SLOS is not known accurately. The relative frequencies of SLOS mutations differ among populations. The estimate range may vary due to different criteria used in the selection of the patients. Although the absolute incidence in some countries is uncertain, it's clear that there are different incidences among various ethnic groups.

#### P09.054

##### Molecular profile of neurodevelopment in trisomy 21 using iPS cells

*M. Sobol<sup>1</sup>, J. Klar<sup>1</sup>, J. Nordlund<sup>2</sup>, G. Annerén<sup>3</sup>, A. Falk<sup>3</sup>, N. Dahl<sup>1</sup>,*

<sup>1</sup>Uppsala University, Uppsala, Sweden, <sup>2</sup>SNP&SEQ Technology Platform, Uppsala, Sweden, <sup>3</sup>Karolinska Institute, Stockholm, Sweden.

We have created an in vitro system for the recreation of key events during early stages of neurodevelopment in Down syndrome (DS) using induced pluripotent stem (iPS) cells. Fibroblasts from two non-related DS patients with trisomy 21 and from two healthy control individuals were reprogrammed to iPS cells followed by differentiation to early neuronal progenitor cells (NPCs) and further into neurons.

Transcriptome profiles were obtained from total RNA of NPCs and neurons. We used the Illumina platform (SNP&Seq platform, SciLifeLab, Uppsala University) and the TopHat and Cufflinks workflow (reference GRCh37). Over 50000 mapped transcripts were identified for each RNA sample.

Data were analysed for differentially expressed transcripts in DS vs. control cells using Cuffdiff and FDR-adjusted p-value of the test statistic (q-values) with a significance cut-off  $q < 0.05$ . We detected 541 and 277 transcripts that were differentially expressed in NPCs and neurons, respectively. Moreover, 221 differentially expressed transcripts were "shared" between both cells types. To clarify pathways and molecular networks in neuronal cells associated with trisomy 21 we used PANTHER and GO analysis. Top GO molecular function clusters were extracellular matrix structural constituent (GO:0005201), receptor activity (GO:0004872) as well as transmembrane transporter activity (GO:0022857) in both NPCs and differentiated neuronal cells.

Our preliminary data suggest that both NPCs and neurons with trisomy 21 show similarities in their transcriptome aberrations when compared to controls. Data are being validated and integrated with genome-wide methylome as well as proteome data in search for markers suitable for rescue-screening.

#### P09.055

##### Mutation in CEP63 co-segregating with developmental dyslexia in a Swedish family

*E. Einarsdottir<sup>1,2</sup>, I. Svensson<sup>3</sup>, F. Darki<sup>4</sup>, M. Peyrard-Janvid<sup>4</sup>, J. M. Lindvall<sup>1,5,6</sup>, A. Ameur<sup>7</sup>, C. Jacobsson<sup>8</sup>, T. Klingberg<sup>4</sup>, J. Kere<sup>1,2</sup>, H. Matsson<sup>1</sup>,*

<sup>1</sup>Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden,

<sup>2</sup>Folkhälsan Institute of Genetics, and Molecular Neurology Research Program, University of Helsinki, Helsinki, Finland, <sup>3</sup>Department of Psychology, Linnaeus University, Växjö, Sweden, <sup>4</sup>Department of Neuroscience, Karolinska Institutet, Solna, Sweden,

<sup>5</sup>Bioinformatics Infrastructure for Life Sciences, Stockholm University, Stockholm, Sweden, <sup>6</sup>Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, <sup>7</sup>Uppsala Genome Center, Uppsala University, Uppsala, Sweden, <sup>8</sup>Department of Pedagogy, Linnaeus University, Växjö, Sweden.

Genetic studies of developmental dyslexia (DD) have presented candidate genes based on mapping of chromosomal translocations, genome-wide linkage and association, and sequencing. Although genome-wide association studies have pinpointed new loci for DD, potential functional gene variants are missing. In contrast, family-based studies continue to be informative in DD, where the full set of variants from individuals can be ascertained in families with a dominant inheritance pattern.

Recently, we used whole exome sequencing (WES) on DNA from seven affected and three unaffected individuals from a Swedish pedigree segregating DD. High quality single nucleotide variants were filtered to retain rare non-synonymous variants segregating with DD but absent from an in-house da-

tabase of WES variants. We identified a predicted damaging mutation resulting in a p.R229L substitution in the centrosomal protein 63kDa (CEP63) required for normal centriole duplication and cell cycle progression. Further, a common CEP63 variant showed significant association with white matter volume partly overlapping with regions influenced by polymorphisms in the DD susceptibility genes *DYX1C1* and *KIAA0319*, consistent with previous reported roles in neuronal migration.

Preliminary confocal imaging showed localisation of both endogenous wild type CEP63 and exogenous p.R229L CEP63 to the centrosome in RPE1 cells suggesting a potential dominant negative effect of the mutant protein. Further studies may reveal the impact of p.R229L CEP63 on cellular function and brain development.

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#### P09.056

##### Mono-allelic and bi-allelic variants in EMC1, implicated in ER-mitochondria communication, are associated with neurodegeneration

*Y. Bayram<sup>1</sup>, T. Harel<sup>1</sup>, G. Yesil<sup>2</sup>, Z. Coban-Akdemir<sup>1</sup>, W. L. Charng<sup>1</sup>, E. Karaca<sup>1</sup>, A. A. Asmar<sup>3</sup>, M. K. Eldomery<sup>1</sup>, J. V. Hunter<sup>4</sup>, S. N. Jhangiani<sup>1,5</sup>, J. A. Rosenfeld<sup>1,6</sup>, D. Pehlivan<sup>1</sup>, A. W. El-Hattab<sup>7</sup>, M. A. Saleh<sup>8</sup>, C. A. LeDuc<sup>9</sup>, D. Muzny<sup>5</sup>, E. Boerwinkle<sup>5,9</sup>, Baylor-Hopkins Center for Mendelian Genomics, R. A. Gibbs<sup>1,5</sup>, W. K. Chung<sup>10</sup>, Y. Yang<sup>1,6</sup>, J. W. Belmont<sup>11</sup>, J. R. Lupski<sup>1,5,11</sup>,*

<sup>1</sup>Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>2</sup>Department of Medical Genetics, Bezmialem University, Istanbul, Turkey, <sup>3</sup>Section of Medical Genetics, Children's Specialist Hospital, King Fahad Medical City, Riyadh, Saudi Arabia, <sup>4</sup>Department of Pediatric Radiology, Texas Children's Hospital, Houston, TX, United States, <sup>5</sup>Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, United States, <sup>6</sup>Baylor Miraca Genetics Laboratories, Baylor College of Medicine, Houston, TX, United States, <sup>7</sup>Division of Clinical Genetics and Metabolic Disorders, Department of Pediatrics, Tawam Hospital, Al-Ain, United Arab Emirates, <sup>8</sup>Departments of Pediatrics, Columbia University Medical Center, New York, NY, United States, <sup>9</sup>Human Genetics Center, University of Texas Health Science Center, Houston, TX, United States, <sup>10</sup>Departments of Pediatrics and Medicine, Columbia University Medical Center, New York, NY, United States, <sup>11</sup>Department of Pediatrics, Baylor College of Medicine, Houston, TX, United States.

Introduction: The paradigm of a single gene associated with one specific phenotype and mode of inheritance has been repeatedly challenged. Genotype-phenotype correlations can often be traced to different mutation types, localization of the variants in distinct protein domains, or the trigger of or escape from nonsense mediated decay.

Materials and Methods: Whole exome sequencing (WES) was applied to four affected individuals in two unrelated families with reported consanguinity, and two other families were sequenced with WES by a trio (proband and parents) approach.

Results: We identified homozygous variants in EMC1 that segregated with a phenotype of developmental delay, hypotonia, scoliosis and cerebellar atrophy in three families. In addition, a de novo heterozygous EMC1 variant was observed in an individual with a similar clinical and MRI imaging phenotype.

Conclusions: EMC1 encodes a member of the endoplasmic reticulum (ER)-membrane protein complex (EMC), an evolutionarily conserved complex which has been proposed to play multiple roles in ER-associated degradation (ERAD), ER-mitochondria tethering, and proper assembly of multi-pass transmembrane proteins. Perturbations of protein folding and organelle crosstalk have been implicated in neurodegenerative processes including cerebellar atrophy.

Monoallelic and biallelic inheritance have previously been associated with different genes in which either de novo mutations (i.e., GJB2, KIF1A, MAB21L2, and NALCN) or recessive mutations (i.e., AARS, CLCN1, COL6A1, DEAF1, EGR2, ENPP1, KRT14, and ROR2) may cause more severe phenotype. We propose EMC1 as a novel gene in which either biallelic or monoallelic variants may lead to a syndrome including intellectual disability and preferential degeneration of the cerebellum.

#### P09.057

##### Novel mutation in CACNA1A gene expressed as myoclonic epilepsy with drop attacks associated with mild cerebellar signs

*G. Larom-Khan, A. Peleg, A. Harari-Shaham, L. Shamer-Meiri, N. Zelnik; Carmel medical center, Haifa, Israel.*

Introduction: A toddler with global developmental delay and ataxia presented at two months of age with myoclonic jerks which later on evolved into drug-resistance myoclonic epilepsy with drop attack.

Methods: Whole exome sequencing was employed and revealed one hete-

rozygous mutation. This alteration was not present in the DNA samples of her parents.

Results: We detected a novel missense mutation (C.678G>T) in the CACNA1A gene. No single nucleotide polymorphisms were found in this region and its probability score was nearly 1.0. The CACNA1A gene encodes the alfa1-subunit A, a transmembrane pore-forming subunit of the P/Q or CaV2.1 voltage gated calcium channel which mediated entry of Ca(2+) ions into excitable cells and also involved in a Ca(2+) dependent processes, including muscle contraction, hormone or neurotransmitter release and gene expression.

Conclusion: CACNA1A mutations were previously reported in Episodic ataxia type2, Familial hemiplegic migraine type 1 and in Spinocerebellar ataxia 6. It was also recently associated with epileptic encephalopathy. The present report expands the clinical spectrum of CACNA1A-associated neurological and particular epileptic disorders, and suggests that early disturbance of the voltage-gated calcium channel activity may underlies neuronal insult which lead to ataxia to myoclonic epilepsy.

## P09.058

### Epileptic seizures associated with chromosomal abnormalities

**M. Budisteanu**<sup>1,2</sup>, A. Tutulan-Cunita<sup>1</sup>, S. Papuc<sup>1</sup>, C. Iliescu<sup>2</sup>, C. Burloiu<sup>2</sup>, I. Focsa<sup>1</sup>, D.

Craiu<sup>2</sup>, I. Minciuc<sup>2</sup>, D. Barca<sup>2</sup>, B. Budisteanu<sup>2</sup>, M. Cristea<sup>1</sup>, I. Borcan<sup>1</sup>, A. Arghire<sup>1</sup>,

<sup>1</sup>Victor Babes National Institute of Pathology, Bucharest, Romania, <sup>2</sup>Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Bucharest, Romania, <sup>3</sup>Titu Maiorescu University, Bucharest, Romania.

Many chromosomal abnormalities are associated with different neurological conditions, including epilepsy. There are about 400 different chromosomal imbalances described with seizures or EEG abnormalities. In this paper we present our experience regarding epilepsy associated with chromosomal abnormalities.

Methods: 125 children with epileptic seizures, other neurological features, and/or dysmorphic features were evaluated by clinical, dysmorphological, neurological, psychiatric and psychological examinations, EEG, neuroimaging studies (CT, MRI), biological studies, genetic investigations (karyotype, FISH, array CGH).

Results: In 42 cases with epilepsy a chromosomal syndrome was identified, including Down syndrome (4 children), trisomy 18 (1 child), partial trisomy 13 (1 child), 1p36 deletion syndrome (3 children), Angelman syndrome (20 children), Miller-Dieker syndrome (2 children), Pallister-Killian syndrome (3 children), Williams-Beuren syndrome (1 child), Wolf-Hirschhorn syndrome (1 child). We identified, also, some very rare chromosomal rearrangements, with complex phenotypes associating epilepsy, which included 3p26 duplication; 8p21.2-p11.2 deletion; 3q26.31 duplication.

Discussion: Most of our patients presented chromosomal syndromes, either known to associate epilepsy in most patients (such as Angelman syndrome, Miller-Dieker syndrome, trisomy 13 and 18) or rarely presenting with seizures (such as Down syndrome, Williams-Beuren syndrome). Several rare chromosomal rearrangements were detected; the genotype-phenotype correlations were difficult to establish in some cases (no specific genes for epilepsy are known to be located in the affected regions - 3p26.3, 3q26.31). Further studies are needed to understand the mechanism of epilepsy associated with chromosomal abnormalities.

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## P09.059

### Targeted gene testing of 50 patients with epilepsy-related neurodevelopmental disorder.

**M. Martinez-Garcia**, M. Peña-Vilabelda, P. Maietta, J. Botet, S. Alvarez; NGS Laboratory. NIMGenetics, Madrid, Spain.

Background: Epilepsy is a common clinical and genetic heterogeneous neurological disorder, with a large number of cases caused by genetic factors. To understand the molecular basis of epilepsy, 141 genes associated to neuro-metabolic disorders, syndromic and non-syndromic epilepsy were routinely analysed on 50 cases.

Methods: Genomic libraries were generated using the Ion AmpliSeqTM Exome RDY as exome backbone, combined with an AmpliSeq panel design to improve gene coverage. Sequencing reads generated on the Ion ProtonTM and Ion S5TM platform were analyzed using Torrent Suite software. Annotated variants using ION Reporter were prioritized with an in-house analytical pipeline to identify the causative variants. This approach assures an average depth of 100X and an average 98.3% coverage in the selected genes.

Results: Among the 50 probands analyzed, 22 (44%) were referred as Early Infantile Epileptic Encephalopathies (EEIE). This approach allowed us to identify potentially genetic causative variants on 40% of the cases. Among EEIE patients 64% (14/22) were genetically diagnosed. Out of the 63 iden-

tified variants, 26 were associated to a gene with an autosomal-dominant inheritance pattern, 5 of which were confirmed as "de novo". Furthermore, a combined analysis of genes related to other neurodevelopmental disorders as autism was performed on 8 cases.

Conclusions: Epilepsy panels based on WES provide a cost effective and comprehensive strategy, that accelerates the identification of a definitive clinical diagnosis, decreasing the familial anxiety, improving the prognosis accuracy and facilitating the therapy selection.

## P09.060

### Detection of genetic heterogeneity in Bulgarian patients with epilepsy

**L. T. Balabanski<sup>1</sup>, S. Ivanov<sup>1</sup>, R. Vazharova<sup>1</sup>, A. Savov<sup>2</sup>, M. Malinov<sup>1</sup>, D. Toncheva<sup>1,3</sup>**

<sup>1</sup>Malinov Clinic, Sofia, Bulgaria, <sup>2</sup>National Genetic Laboratory, Sofia, Bulgaria,

<sup>3</sup>Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria.

Background: Epilepsy encompasses a group of related neurological disorders characterised by recurrent seizures due to abnormal electric activity in the brain. The genetic basis of the disease is complex with defects in more than 200 genes predisposing to it. The aim of our study was to perform targeted next-generation sequencing and detect gene defects in 12 Bulgarian patients with either primary (6) or secondary epilepsy as part of another undetermined condition (6).

Methods: After DNA was extracted from blood, NGS libraries were prepared using either TruSight One or TruSight Inherited Disease gene panels. Sequencing was performed on an Illumina MiSeq system. Variant filtering and downstream analysis was done using Variant Studio and GenomeBrowse softwares.

Results: We detected pathogenic mutations in 6 of our patients (50%). Three of those with primary epilepsy carried missense mutations in SCN8A (p.Arg1872Gln), EFHC1 (p.Phe229Leu) and a deletion in CLCN1 (c.1436\_1449delTACCTGCGGAGGC). The genes affected in the patients with secondary epilepsy were RNASEH2B (p.Ala177Thr), IDS (p.Pro408Ser) and AP1S2 (p.Tyr86Ter), and their carriers were respectively diagnosed with Aicardi-Goutieres syndrome, mucopolysaccharidosis and X-linked syndromic mental retardation.

Discussion: The genetic heterogeneity in our cohort was expected bearing in mind the wide variability in severity and penetrance of the disorders from the epileptic spectrum. This underlines the need for using large gene panels in order to successfully detect defects in patients with epilepsy as a symptom. The identification of the causative mutation could deliver crucial insights into the aetiology of the disease and potentially pave the way for a personalised treatment.

## P09.061

### Dogs Reveal a Novel Candidate Gene for Human Myoclonic Epilepsies

**R. Sarviah<sup>1,2</sup>, F. Wieländer<sup>3</sup>, F. James<sup>4</sup>, M. Cortez<sup>5</sup>, G. Kluger<sup>6,7</sup>, M. Kornberg<sup>8</sup>, A. Bathen-Noethen<sup>9</sup>, T. Flegel<sup>10</sup>, S. Bhatti<sup>11</sup>, M. Arumilli<sup>11,2</sup>, M. Hytönen<sup>1,2</sup>, A. Fischer<sup>9</sup>, H. Lohi<sup>1,2</sup>**

<sup>1</sup>Research Programs Unit, Molecular Neurology and Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, <sup>2</sup>Folkhälsan Research Center, Helsinki, Finland, <sup>3</sup>Centre for Clinical Veterinary Medicine, LMU University of Munich, Munich, Germany, <sup>4</sup>Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Ontario, ON, Canada, <sup>5</sup>Neurosciences & Mental Health Program, Peter Gilgan Centre for Research and Learning, SickKids Research Institute, Ontario, ON, Canada,

<sup>6</sup>Department of Neuropediatrics, Epilepsy Center, Vogtareuth, Germany, <sup>7</sup>PMU Salzburg, Salzburg, Austria, <sup>8</sup>Veterinary Hospital Trier, Trier, Germany, <sup>9</sup>Veterinary Practice Bathen-Noethen, Cologne, Germany, <sup>10</sup>Department of Small Animal Medicine, University of Leipzig, Leipzig, Germany, <sup>11</sup>Department of Medicine and Clinical Biology of Small Animals, Ghent University, Ghent, Belgium.

Introduction: Epilepsy is the most common chronic neurological disease in humans and dogs. Canine epilepsy resembles the human condition and provides opportunity for new gene discovery, functional characterization and establishment of a therapeutic model. We previously identified young dogs with myoclonic epileptic seizures in the Rhodesian Ridgeback (RR) breed (Wieländer et al., JVIM 2016; 30:444) and aimed here to further characterize the clinical and genetic background.

Materials and Methods: A study population of 17 RRs was established and the breed-specific myoclonic epilepsy was studied by a wide-scale medical examination and awake ambulatory wireless video-EEG. A GWAS was performed to map the gene in a cohort of 10 cases and 18 unaffected RRs followed by whole exome sequencing of two cases. Candidate mutation was validated in a larger cohort (n=40) of cases and controls by TaqMan qPCR assays. Epileptic cases across breeds (n= 965) were also tested for the mutation. The expression pattern of the candidate gene was established in 27 canine tissues including many brain regions by RT-PCR.

Results: Our clinical studies revealed a photosensitive myoclonic epilepsy with a characteristic EEG pattern and age of onset in juvenile dogs. Genetic

studies identified a fully penetrant recessive breed-specific truncating mutation in a novel neuronal RasGTPase gene.

Conclusions: Our study revealed a myoclonic epilepsy in juvenile dogs resembling partially human JME. Canine gene discovery represents a novel candidate gene for human myoclonic epilepsies and unravels a new disease mechanism while establishing a clinically relevant animal model for further functional and therapeutic approaches.

## P09.062

### Diagnostic targeted next generation sequencing in patients with epilepsy

P. Rump<sup>1</sup>, Y.J. Vos<sup>1</sup>, D.R. M. Vlaskamp<sup>1,2</sup>, P.M.C. Callenbach<sup>2</sup>, R.J. Sinke<sup>1</sup>, C.M.A. van Ravenswaaij<sup>1</sup>, O.F. Brouwer<sup>2</sup>;

<sup>1</sup>University of Groningen, University Medical Centre Groningen, Department of Genetics, Groningen, Netherlands, <sup>2</sup>University of Groningen, University Medical Centre Groningen, Department of Neurology, Groningen, Netherlands.

Introduction: Epilepsy has a heterogeneous aetiology with a substantial genetic component. We used a targeted next generation sequencing (NGS) gene panel for clinical genetic testing in a heterogeneous cohort of 125 patients with epilepsy.

Materials and Methods: A gene panel, based on Agilent Sure Select Target Enrichment®, including 144 genes was used. Within this panel, nine partly overlapping subpanels were designed based on different epilepsy phenotypes, namely: benign familial neonatal/infantile epilepsy (5 genes), epileptic encephalopathy (39 genes), focal epilepsy (12 genes), fever related epilepsy (12 genes), progressive myoclonic epilepsy (19 genes), metabolic disorders with epilepsy (43 genes), generalized epilepsies (14 genes), epilepsy in combination with other paroxysmal disorders (11 genes), and syndromes with epilepsy and intellectual disability (63 genes). Based on the phenotype of the patient one or more of these subpanels were analysed.

Results: A (likely) pathogenic variant was identified in 12.6% of the patients. All confirmed by Sanger sequencing. The yield was highest in patients with benign familial neonatal/infantile epilepsy (23%) or fever related epilepsy (22%). The genes involved were: CACNA1A, CDKL5, GRIN2A, KCNQ2, KCNQ3, MECP2, NHLRC1, PCDH19 (n=3), PRRT2, RANBP2 (n=2), SCN1A, and SYNGAP1. Using this approach we were also able to detect a mosaic pathogenic variant in the PCDH19 gene in a male patient. A variant of unknown significance was reported in 11.8% of the patients. In most instances clinical relevance could be excluded.

Conclusions: Our results demonstrate that targeted next generation sequencing offers a quick, comprehensive and efficient molecular screening in patients with epilepsy.

## P09.063

### Identification of a de novo mutation in a patient with idiopathic epilepsy by targeted exome sequencing

L. Rausell, M. Lázaro, L. Cabrera, M. Bermejo, M. Molero, A. Gómez, A. Arteche, P. Marín-García, C. Ruiz, J. García-Planells, M. García-Hoyos;

Instituto de Medicina Genómica (IMEGEN), Paterna, Spain.

Introduction: NGS (Next Generation Sequencing) approach is revolutionizing our understanding in medical genetics and is increasingly allowing us to achieve genetic diagnosis of complex disorders. One example of such complex disorders is Epilepsy. Epilepsy is the most common neurological disorder in which nerve cell activity in the brain becomes disrupted, causing seizures or periods of unusual behaviour, sensations, and sometimes loss of consciousness. The genetic diagnosis of Epilepsy is usually very difficult because of the genetic heterogeneity and the high number of genes associated with the disease.

Objective: Genetic characterization of a patient with epilepsy by targeted exome sequencing.

Methods: The targeted exome sequencing for Epilepsy is encompassing all the coding regions of 116 genes associated with the disease. Library was prepared using TruSight One (Illumina) and samples were ultrasequenced using a NextSeq 500 sequencing system (Illumina).

Non-benign variants were confirmed and segregated in parental samples by Sanger sequencing.

Results: The use of this targeted exome in a patient with idiopathic epilepsy allowed us to identify a de novo probably disease-causing change in the SCNA8 gene.

Conclusion: Targeted exome is a diagnostic alternative to Sanger sequencing for genetic heterogeneous diseases such as Epilepsy. This new strategy gives us the possibility to simultaneously analyse several genes, reducing time and cost, and increasing the diagnostic yield.

Early and accurate diagnosis of patients with epilepsy is essential for suc-

cessful treatment and care, especially at the evolutionary phases of the disease.

## P09.064

### Exome sequencing for molecular analysis of early infantile epileptic encephalopathies (EIEE)

A. Marçé-Grau<sup>1</sup>, E. Cuenca-León<sup>1</sup>, A. Felipe-Rucián<sup>1</sup>, F. Lopez-Pisón<sup>2</sup>, M. Raspall-Chaure<sup>1</sup>, A. Macaya<sup>1</sup>;

<sup>1</sup>Vall d'Hebron Institut de Recerca, Barcelona, Spain, <sup>2</sup>Hospital Universitario Miguel Servet, Zaragoza, Spain.

Introduction: Genetic variants causing EIEE are currently identified in only a minority of cases. We explored the usefulness of Whole-Exome-Sequencing (WES) for both diagnosis and gene discovery in EIEE.

Methods: Peripheral blood DNA was extracted from forty unrelated trios (proband and unaffected parents). We used NimbleGen SeqCap EZ Exome v3.0 and Illumina TruSeq kits to generate libraries which were 30X sequenced in a HiSeq 2000 instrument. Exomes were aligned to NCBI build 37 reference sequence. Variants were called with SamTools, annotated using SnpEff and VarAnnot and classified according to predicted functional effect. Hard filters and IGV inspection were used to eliminate artifacts. Analysis was two-tiered and comprised evaluation of 169 epilepsy genes, followed by evaluation of remaining WES variants. Selected variants were Sanger-validated.

Results: A first-tier analysis revealed high-effect de novo mutations in EIEE-associated genes in 45% of the cases. STXBP1, KCNQ2, KCNT1 and SCN1A were the most commonly mutated genes in this cohort. In patients with neonatal presentation, WES displayed a higher diagnostic yield, 66% (12/18; p<.01). Second-tier analysis showed an excess of damaging mutations in several intolerant-to-variation genes, mainly related to synaptic function and neural transmission.

Conclusion: WES analysis led to the identification of de novo disease-causing mutations in genes previously related to EIEE in 45% of our cases, showing a better diagnostic yield in cases with neonatal onset. Among the remaining undiagnosed cases, several de novo variants emerged as plausible candidates to produce EIEE. Functional ongoing studies will help determining their causative role in the disease.

## P09.065

### A novel mutation in PCDH19 enabled genetic diagnosis as Epilepsy and Mental Retardation Limited to Females

E.N. Tuncer<sup>1</sup>, S.A. Ugur Iseri<sup>1</sup>, B. Kara<sup>2</sup>, H. Maras Genc<sup>2</sup>, E. Uyur Yalcin<sup>2</sup>, U. Ozbek<sup>1</sup>;

<sup>1</sup>Istanbul University, Institute of Experimental Medicine Department of Genetics, Istanbul, Turkey, <sup>2</sup>Kocaeli University Faculty of Medicine, Department of Pediatrics, Division of Child Neurology, Kocaeli, Turkey.

Introduction: Epilepsy and Mental Retardation Limited to Females (EFMR) is a rare encephalopathy associated with PCDH19 mutations affecting heterozygous females, sparing hemizygous males. EFMR is characterized by intractable, fever sensitive generalized or focal seizures initiating within the first year of life, and clustered brief seizures occurring repeatedly during several days. This study includes a 4-year-old girl, whose genetic evaluation aided differential diagnosis between EFMR and Dravet syndrome (DS).

Materials and Methods: A 4-year-old girl with prominent intellectual disability was recruited for this study. Her physical, neurological, neuroimaging and electroencephalography (EEG) examinations were performed with information on family history. Her first seizure occurred at 8 months of age with fever, marking the beginning of neuromotor developmental delay. Atypical absence seizures emerged when she was 1.5 years old. She is the only affected person in the family among 2 brothers and 1 sister. Illumina HiSeq2000 was utilized in whole genome exome sequencing of the subject. Exome data was filtered to detect all genetic variants in DS related genes including PCDH19. Candidate genetic variants were subjected to familial segregation through PCR and direct sequencing.

Results: Genetic analyses revealed a novel nonsense mutation in PCDH19 apparently occurring de novo. In silico evaluations supported mutation's role in pathogenesis.

Conclusion: This study enabled genetic diagnosis of the case as EFMR, associating a novel PCDH19 mutation with the disease that provided the family with genetic consulting.

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## P09.066

**Progressive Cerebello-Cerebral Atrophy (PCCA) and Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy (PEHO) are allelic syndromes**

D. Lev<sup>1,2</sup>, R. Hady-Cohen<sup>1</sup>, H. Ben-Pazi<sup>3</sup>, E. Leshinsky-Silver<sup>4</sup>, T. Lerman-Sagie<sup>1</sup>;  
<sup>1</sup>Wolfson Medical Center, Holon, Israel, <sup>2</sup>Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel, <sup>3</sup>Shaare Zedek Medical Center, Jerusalem, Israel.

In 2003, a new syndrome was diagnosed in the Sephardic Jews population, it was named PCCA (Progressive Cerebello-Cerebral Atrophy) after its typical neuroradiological findings. There are two types: PCCA type 1 and PCCA type 2. Known causative genes are SEPSECS in type 1 and VPS53 in type 2. PEHO (Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy) is a syndrome prevalent in Finland. It includes progressive microcephaly, profound mental retardation, marked hypotonia and dysmorphic features. MRI shows progressive cerebellar and brainstem atrophy. Recently, mutations SEPSECS were found in patients with PEHO.

We diagnosed 2 siblings with PEHO. They have a developmental encephalopathy, limb and facial edema, infantile spasms and optic atrophy. MRI shows cerebellar and brainstem atrophy. Extensive workup did not reveal the causative gene. Whole exome sequencing was performed. The first analysis that was done 5 years ago did not reveal the causative mutations. Recently, a re-analysis of the data revealed the 2 common Moroccan mutations in VPS53 (exon 19 c.2084A>G p.(Gln695Arg) and c.1556+5G>A. The parents were found to be carriers of the mutations.

Comparing both syndromes reveals clinical and radiological similarities. Our revelation that VPS53 mutations, previously described to be the cause of PCCA1, and now are found as causative mutations is PEHO, actually put these two syndromes on the same spectrum. Likewise, SEPSECS gene that is the cause for PCCA1 was also found recently as the causative gene in PEHO. PCCA and PEHO share the same genetic and clinical spectrum and thus, are allelic syndromes.

## P09.067

**A polymorphism in CRAT is associated with HLA-DQB1\*06:02 negative essential hypersomnia**

T. Miyagawa<sup>1</sup>, S. Khor<sup>2</sup>, H. Toyoda<sup>2</sup>, M. Yamasaki<sup>2</sup>, M. Shimada<sup>2</sup>, K. Mishima<sup>3</sup>, Y. Honda<sup>3</sup>, K. Tokunaga<sup>2</sup>, M. Honda<sup>1</sup>;

<sup>1</sup>Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, <sup>2</sup>The University of Tokyo, Tokyo, Japan, <sup>3</sup>Neuropsychiatric Research Institute, Tokyo, Japan.

Essential hypersomnia (EHS) is a sleep disorder characterized by excessive daytime sleepiness but no cataplexy, can be divided into two broad classes based on the presence or absence of HLA (human leukocyte antigen)-DQB1\*06:02 allele. HLA-DQB1\*06:02 positive EHS and narcolepsy are associated with the same susceptibility genes. However, there are fewer studies of HLA-DQB1\*06:02 negative EHS. Therefore, we performed a genome-wide association study in 119 Japanese patients with HLA-DQB1\*06:02 negative EHS and 1,582 Japanese healthy individuals to identify susceptibility genes associated with HLA-DQB1\*06:02 negative EHS. A replication study was conducted on 191 Japanese patients with HLA-DQB1\*06:02 negative EHS and 433 Japanese healthy individuals. SNP rs10988217 located in CRAT (carnitine acetyltransferase) was found to be significantly associated with HLA-DQB1\*06:02 negative EHS ( $P < 5 \times 10^{-8}$ , OR=2.8). An eQTL analysis showed that rs10988217 was significantly correlated with expression levels of CRAT in various tissue or cell types, including brain tissue ( $P < 0.05$ ). CRAT gene encodes the carnitine acetyltransferase protein, which is a key enzyme for metabolic pathways involved with the control of the acyl-CoA/CoA ratio in mitochondria, peroxisomes and endoplasmic reticulum. In addition, The Metabolomics GWAS Server (doi: 10.1038/ng.2982) revealed that rs10988217 affected succinylcarnitine levels in blood ( $P < 10^{-17}$ ). Individual acylcarnitines levels were measured in 36 Japanese patients with HLA-DQB1\*06:02 negative EHS and 68 Japanese healthy individuals. Levels of several acylcarnitines showed significant differences between the two groups. The results provide evidence that HLA-DQB1\*06:02 negative EHS may have an underlying dysfunction in fatty acid oxidation pathway.

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## P09.069

**Are rare coding mutations in the genes related to genetic peripheral neuropathies risk factors in multiple sclerosis (MS)**

A. Peterlin<sup>1</sup>, A. Maver<sup>1</sup>, A. Hodžić<sup>1</sup>, Š. Šega<sup>2</sup>, J. Drulović<sup>3</sup>, I. Novaković<sup>4</sup>, T. Pekmezović<sup>5</sup>, S. Ristić<sup>6</sup>, M. Kapović<sup>7</sup>, B. Peterlin<sup>1</sup>;

<sup>1</sup>Clinical Institute of Medical Genetics, Ljubljana, Slovenia, <sup>2</sup>Clinical Institute of Medical Genetic, UMC Ljubljana, Ljubljana, Slovenia, <sup>3</sup>Neurology Clinic, CCS, Beograd, Belgrade, Serbia, <sup>4</sup>Institute of Human Genetics, MF Beograd, Belgrade, Serbia, <sup>5</sup>Department of

**Biology and Medical Genetics, School of Medicine Rijeka, Rijeka, Croatia.**

Introduction: MS is chronic inflammatory disease of the central nervous system with important genetic contribution. Although familial contribution to MS etiology is well established, much of genetic contribution still remains poorly defined. Several case reports reported comorbidity between different types of genetic peripheral neuropathies and MS. Therefore we hypothesized that there is an increased mutation burden in genes related to peripheral neuropathies among MS patients.

Materials and Methods: Whole exome sequencing using Nextera Coding Exome enrichment was performed in 48 patients with familial MS, 40 patients with sporadic MS and 92 population-matched controls. Genotypes were called using GATK toolkit in multisample mode and only sites with variant quality over 100.0 and genotyping rate of over 60% across all samples were kept in downstream analyses. The selection of variants among bioinformatically focused panel of 52 peripheral neuropathy related genes was narrowed in accordance of functional impact predicted by snpEff - all truncating variants and missense variants predicted as pathogenic by a majority of in-silico predictors, were considered in further burden analyses.

Results: We identified 8 candidate genetic variants (7 genes) that affect function in familial MS, and 11 candidate genetic variants (12 genes) in sporadic MS patients. Overall we detected a statistically significant 1.9-times enrichment of mutations ( $p=0.004$ ) in the familial MS cases compared to the ExAC control samples.

Conclusions: Exome sequencing of peripheral neuropathy causing genes revealed an excess burden of deleterious coding variants in familial MS patients which supports previous evidence of comorbidity among peripheral neuropathies and MS.

## P09.070

**Familiality of cognitive performance: analysis in patients with psychotic disorders and their first degree relatives**

C. Prats<sup>1</sup>, P. Ferentinos<sup>2</sup>, J. Soler<sup>3</sup>, S. Miret<sup>4</sup>, S. Campanera<sup>5</sup>, M. Giralt<sup>6</sup>, M. Muñoz<sup>7</sup>, L. Fañanás<sup>1</sup>, M. Fatjó-Vilas<sup>1</sup>;

<sup>1</sup>Faculty of Biology, University of Barcelona, Barcelona, Spain; Biomedicine Institute of the University of Barcelona (IBUB). Centre for Biomedical Research Network on Mental Health (CIBERSAM), Barcelona, Spain, <sup>2</sup>2nd Department of Psychiatry, University of Athens, Greece, Spain, <sup>3</sup>Facultat de Biologia, Universitat de Barcelona. Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain, <sup>4</sup>Servei de Salut Mental, Psiquiatria i Addiccions, Hospital de Santa Maria. Institut de Recerca Biomedica (IRB), Lleida, Spain, <sup>5</sup>Centre de Salut Mental de Lleida, Servei de Salut Mental i Addiccions, Hospital Santa Maria, Lleida, Spain, <sup>6</sup>Àrea d'Adolescents, Complex Assistencial en Salut Mental, Benito Menni. Sant Boi de Llobregat, Barcelona, Spain, <sup>7</sup>Àrea d'Adolescents. Complex Assistencial en Salut Mental, Benito Menni. Sant Boi de Llobregat, Barcelona, Spain.

Background: Cognitive impairment can be observed not only in psychotic patients but also in their nonpsychotic relatives which highlight a familial component for the relationship between cognition and psychosis. However, not many studies have explored the degree of intrafamilial resemblance of cognitive deficits. Our aim was to examine the familial aggregation of several neurocognitive traits in a sample of patients with psychotic disorders and their first-degree relatives.

Methods: Familiality was explored in 71 patients and 153 healthy first-degree relatives. All underwent a neurocognitive-battery (IQ-WAIS, phonemic/semantic fluency, WCST). Family-level residual intraclass correlation coefficient (ICC) was calculated. Families were ranked according to the level of familiality (IRS) for each cognitive test. Analyses were implemented with Stata v.13.

Results: Significant familial aggregation was found for Intelligence quotient (IQ), phonemic and semantic fluency (executive function domain) ( $p < 0.001$ ). Significant IRS was obtained for IQ and phonemic fluency ( $p < 0.001$ ), classifying these families according to their familiality level. No correlation was found between IRS and their neurocognitive scores ( $p > 0.05$ ), meaning that familial similarities among families are independent of the neurocognitive performance.

Discussion: Despite that different studies have found that patients and relatives display deficits on a variety of neuropsychological tasks, we detected that only IQ, phonemic and semantic fluency show a familial aggregation pattern. This is in line with the fact that even the cognitive heterogeneity of the illness, executive dysfunction is one of the most marked cognitive impairments in SZ (Snitz et al., 2006). In this sense, stratifying families according their familiality levels may reduce heterogeneity and facilitate the identification of shared genes/environmental factors involved.

## P09.072

### FOXP1 and NOVA1 haploinsufficiency in an infant with microcephaly, seizures and severe neurodevelopmental delay

H. Fryssira<sup>1</sup>, S. Psomi<sup>1</sup>, E. Tsoutsou<sup>1</sup>, S. Amenta<sup>2</sup>, E. Manolakos<sup>3</sup>, I. Papoulidis<sup>3</sup>, S. Kitsiou-Tzeli<sup>1</sup>, N. Chaliasos<sup>4</sup>;

<sup>1</sup>Medical Genetics, School of Medicine, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece, <sup>2</sup>"Mitera" Maternity Hospital, Athens, Greece, Athens, Greece, <sup>3</sup>Access to Genome" Clinical Laboratory Genetics, Athens, Greece, <sup>4</sup>Child Health Department, University Hospital of Ioannina (UHI), Ioannina, Greece.

**Introduction:** FOXG1 gene mutations have been associated with the congenital variant of Rett syndrome (RTT) since the initial description of two patients in 2008. The ongoing accumulation of clinical data suggests that the FOXG1-variant of RTT forms a distinguishable phenotype, consisting of mainly congenital microcephaly, seizures, hypotonia, developmental delay and corpus callosum agenesis.

**Case Report:** We report a 6-month-old female infant, born at 38 weeks of gestation after in vitro fertilization, who presented with feeding difficulties, irritability and developmental delay since birth. Microcephaly, a small forehead with bitemporal narrowing, dyspraxia, poor eye contact and strabismus were also noted. At 10 months the proband exhibited focal seizures and required valproic acid treatment. Sleep deprivation EEG showed signs of focal cerebral dysfunction. The MRI was normal.

The peripheral blood G-banding karyotype was normal (46, XX), as well as the DNA analysis of the MECP2 gene. Array-Comparative Genomic Hybridization was performed (AGILENT Technologies, resolution 200 Kb) and revealed a 4.09 Mb loss of the copy numbers in the spanning region 14q12, encompassing the FOXG1 and NOVA1 genes.

**Conclusions:** The proband presented similar features with patients with 14q12 deletions except for dysgenesis of corpus callosum. Disruption of the NOVA1 gene, which promotes the motor neurons apoptosis, has not been yet linked to any human phenotypes, but could act synergistically with the FOXG1 gene in our patient.

Since our patient is the first reported case with deletions of both the above genes, the thorough clinical follow-up could further delineate the Congenital Rett-Variant phenotypes.

## P09.074

### Molecular diagnosis improvement in patients with frontotemporal dementia using Next-Generation Sequencing

A. de la Hoz<sup>1,2</sup>, E. Blanco<sup>3,4</sup>, N. Ibarluzeta<sup>1</sup>, R. Bereircua<sup>2</sup>, A. Molano<sup>2</sup>, I. Ugarriza<sup>2</sup>, S. del Mazo<sup>3</sup>, M. A. López-Ariztegui<sup>3</sup>, M. Fernández<sup>3,1</sup>, M. I. Tejada<sup>1,3,2</sup>;

<sup>1</sup>BioCruces Health Research Institute, Barakaldo, Spain, <sup>2</sup>CIBERER, Madrid, Spain,

<sup>3</sup>Cruces University Hospital, Barakaldo, Spain.

#### Introduction:

Frontotemporal dementia (FTD) is the second most common form of young-onset dementia after Alzheimer's disease and comprises about 10–20% of all dementias worldwide. It is inherited in 30–50% of patients. It includes a group of degenerative diseases characterized by the atrophy of frontal and temporal lobes. Mutations in three main genes are commonly associated with FTD: MAPT, PGRN, and C9orf72, but recent findings have identified rare and pathogenic variants in additional genes: FUS, VCP, TARDBP and CHMP2B. Next Generation Sequencing (NGS) is being used to identify new pathogenic variants.

#### Material and Methods:

A total of 48 FTD patients were assessed by means of a NGS panel. Target regions in the selected genes (MAPT, PGRN, FUS, VCP, TARDBP and CHMP2B) were amplified using an Ion AmpliSeq™ custom Panel. It consists of 93 amplicons (17.21Kb), with coverage close to 99.5% on targeted sequence. Libraries were sequenced on an Ion Torrent PGM platform. Data analysis was performed using Torrent Suite™ and Ion Reporter™ software. All variants were validated by Sanger.

**Results:** Two known pathogenic mutations (c.709-1G>A and c.359C>A) and a non-described missense variant (c.1604G>A) were found in PGRN. We also detected a polymorphic variant in FUS (3'-untranslated region [UTR], c.\*41G>A), whose MAF is 0.005, in three of our patients.

**Conclusion:** This approach shows the utility of the NGS technology to identify both known pathogenic and low frequency variants likely associated with this disorder. It is a powerful and cost-effective tool to diagnose complex disorders with genetic heterogeneity such as FTD.

Gobierno Vasco.

## P09.075

### Carriage of one or two FMR1 premutation alleles seems to have no effect on illness severity in a FXTAS female with an autozygous FMR1 premutation allele

L. Rodriguez-Revenga<sup>1</sup>, J. Pagonabarraga<sup>2</sup>, E. Granell<sup>2</sup>, M. Alvarez-Mora<sup>1</sup>, I. Madrigal<sup>1</sup>, M. Milà<sup>1</sup>;

<sup>1</sup>Hospital Clinic, Barcelona, Spain, <sup>2</sup>Hospital Sant Pau, Barcelona, Spain.

**Introduction:** Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that occurs in FMR1 premutation carriers. The prevalence of FMR1 premutation carriers in the general population is relatively high, and although rare, a premutation in both X chromosomes may occur in females inheriting a premutation allele from each of both parent carriers. Here we report the first female with an autozygous (homozygous by descent) FMR1 premutation allele, who fulfills neurological and radiological FXTAS findings/criteria.

**Material and Methods:** Molecular characterization included CGG repeat length, AGG interruption pattern, FMR1 mRNA, FMRP levels quantification and SNP microarray. Neuroradiological assessment of 3T magnetic resonance imaging, neurological and cognitive/neuropsychological evaluations were performed.

**Results:** Neurological and neuroradiological examination of the female with the same FMR1 allele in the premutation range (77 CGGs) demonstrated FXTAS features. Further familial evaluation showed a similar neuropsychiatric profile, with impairments in cognitive flexibility and visuospatial function, mainly.

**Conclusions:** A unique family with an autozygous FMR1 premutation female is presented. Neurological/cognitive and neuroradiological examinations revealed FXTAS-specific findings in the female with the autozygous FMR1 premutation allele. The consistent molecular and cognitive/psychiatric phenotype in family members suggests that carrying one or two FMR1 premutation alleles has no effect on illness severity.

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## P09.076

### Social anxiety and autism spectrum traits among adult FMR1 premutation carriers

M. Milà<sup>1</sup>, O. Lopez-Moure<sup>2</sup>, E. Mur<sup>3</sup>, I. Madrigal<sup>1</sup>, M. Alvarez-Mora<sup>1</sup>, L. Rodriguez-Revenga<sup>1</sup>;

<sup>1</sup>Hospital Clinic, Barcelona, Spain, <sup>2</sup>PIC, Parc de Recerca Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>3</sup>CSM Martí i Julià. Instituto de Neuropsiquiatría y Adicciones (INAD). Parc de Salut Mar, Barcelona, Spain.

**Introduction:** Behavioral symptoms and traits have been proposed as early markers in neurodegenerative diseases. The aim was of this study was to evaluate social anxiety and autism in FMR1 premutation carriers and controls using the Social Phobia Inventory and the Autism-Spectrum Quotient. **Material and Methods:** Fifty-nine premutation carriers (40 females; 19 males) (7 females and 9 males with FXTAS) were compared to 50 controls (32 females; 18 males).

**Results:** Our results show that FMR1 premutation carriers have higher social anxiety scores and autistic traits compared to controls. Indeed, the SPIN scores were higher increased in women FMR1 premutation carriers compared to men whereas the AQ scores were similar between the two genders. Finally, SPIN scores were correlated with the repeat size when the CGG number was greater than 100.

**Conclusions:** Overall, these results indicate that social anxiety is greater in FMR1 premutation carrier women than in controls but not in men. In addition, both men and women with the FMR1 premutation have an increased risk of autism traits. Our results suggest that a wide range of behavioral/psychiatric traits should be included within the Fragile X Spectrum disorder and that the penetrance of these traits depends on the genetic background (CGG repeat number, gene modifiers, epigenetic, among others) as well as on gender.

**Acknowledgments:** This work was supported by the Instituto de Salud Carlos III (ISCIII; PI12/00879), cofinanced by the Fondo Europeo de Desarrollo Regional 'una manera de hacer Europa' and AGAUR from the Autonomous Catalan Government (2014 SGR603).

## P09.077

**Skewed X inactivation in women carrying the FMR1 premutation and its relation with Fragile-X-Associated Tremor/Ataxia Syndrome**

M. Alvarez-Mora, L. Rodriguez-Revenga, A. Feliu, C. Badenas, I. Madrigal, M. Mila; Hospital Clinic, Barcelona, Spain.

**Introduction:** Fragile-X-associated tremor/ataxia syndrome (FXTAS) is a late-onset multisystem neurological disorder characterized by intention tremor and cerebellar ataxia. We hypothesized that in FMR1 premutation females with FXTAS, a normal X chromosome might more frequently be inactivated; therefore, the aim of this study was to determine the relationship between skewed X chromosome inactivation (XCI) and FXTAS.

**Material and Methods:** We studied the XCI patterns of cases of FMR1 premutation in 10 women with FXTAS and 21 without FXTAS.

**Results:** The distribution of XCI patterns in the FXTAS and no-FXTAS groups showed differences regarding the allele presenting severe skewed XCI. In the FXTAS group, all cases preferentially inactivated the non-expanded X chromosome, whereas in the no-FXTAS group, all inactivated the expanded X chromosome. As expected, we found statistically significant differences in the skewed XCI on comparing FMR1 premutation women and controls.

**Conclusion:** Although the reduced sample size and blood XCI patterns are two limitations of this study, our results suggest that the skewed XCI of the normal FMR1 allele may be a risk factor for the development of FXTAS. Furthermore, our findings also support the protective effect of the expression of a normal FMR1 allele.

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## P09.078

**Neonatal onset epilepsy and polymicrogyria associated with GRIN1 mutation**

E. Banne<sup>1,2</sup>, L. Shemer- Meiri<sup>3</sup>, N. Zelnik<sup>3</sup>, V. Meiner<sup>4</sup>;

<sup>1</sup>Center for Clinical Genetics, Hadassah-Hebrew University Hospital, Jerusalem, Israel,

<sup>2</sup>Genetic Institute, Kaplan Medical Center, Clalit Health Services, Rehovot, Israel, <sup>3</sup>The Pediatric Neurology Unit, Carmel medical center, Haifa, Israel.

**Introduction:** Severe cases of polymicrogyria are linked with multiple neurological abnormalities, and considered an epileptogenic malformation. We present a child with severe psychomotor retardation, congenital progressive microcephaly, intractable epilepsy presenting as early-onset neonatal seizures, and generalized hypotonia. Brain MRI showed a migration and sulcation defect with bilateral fronto-parietal and temporal polymicrogyria.

**Methods:** Chromosomal microarray and metabolic testing were normal. Whole exome sequencing followed by filtration of synonymous, dbSNP132 and in-house database variants identified a de-novo pathogenic mutation. Result: We detected a novel de-novo heterozygous mutation (c.2365G>A; p.D789N) in the GRIN1 gene. The gene encodes an NMDA receptor subtype of glutamate-gated ion channels with high calcium permeability and voltage-dependent sensitivity to magnesium which is mediated by glycine. NMDA receptors play an important role in the development of the central nervous system, processes of learning, memory and neuroplasticity and are crucial for neuronal communication.

**Conclusions:** The present report expands the clinical spectrum of GRIN1-associated neurological disorders, and supports human NMDA receptors have an in-vivo role in neuronal migration. GRIN1 mutations were previously reported in children with epilepsy, non-syndromic intellectual disabilities and recently with epileptic encephalopathy but not with malformations of cortical migration. Experimental models of NMDA receptor unit-specific knock-down/knock-out show a role for NMDA receptors in cortical neuron migration. Whether the described mutation serves as a dominant negative mutation, perhaps as a stereotactic disruption of the NMDA receptor heterodimerization, or as a toxic gain of function is yet to be investigated.

## P09.079

**Hereditary sensory and autonomic neuropathy type V: study of 2 cases**

W. Manoubi<sup>1</sup>, A. Mili<sup>1</sup>, E. Achouri<sup>2</sup>, A. Gdissa<sup>1</sup>, J. Bouguila<sup>3</sup>, S. Haddad<sup>2</sup>, N. Souayah<sup>3</sup>, S. Sassi<sup>1</sup>, A. Msakni<sup>1</sup>, R. Ben Sghaier<sup>1</sup>, A. Saad<sup>1</sup>, M. Gribbal<sup>1</sup>;

<sup>1</sup>Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology

Farhat Hached Universi, Sousse, Tunisia, <sup>2</sup>Department of Pediatrics, CHU Fattouma Bourguiba University Hospital, Monastir, Monastir, Tunisia, <sup>3</sup>Department of Pediatrics, Farhat Hached University Hospital, Sousse, Tunisia.

**Introduction:** Hereditary sensory and autonomic neuropathy (HSAN)

type 4 is a rare inherited disease caused by a mutation in the neurotrophic tyrosine kinase receptor, type 1 gene located on chromosome 1. It is characterized by pain insensitivity, partial anhidrosis and pressure sensitivity. Self-mutilation injury involving the teeth, lips, ears, eyes, nose, and fingers are invariable feature of this disorder. **Objective:** In this context, we report a Tunisian family having two children affected by HSAN4. Indirect genetic analyzes showed a transmission profile for the HSAN by mutation of the *NTRK1* gene. **Patients and methods:** It is about a Tunisian consanguineous family. It shows two boys aged 6 and 4 years respectively with convulsive recurrent seizures and significant self-harm injuries. A total anhidrosis, insensitivity to pain and mental retardation were then highlighted. Genotyping by analysis of four microsatellite markers surrounding or inside the *NTRK1* gene, helped to establish the haplotype of each individual and to follow the transmission of haplotype associated with the disease. **Results:** the indirect study by genotyping revealed the presence of a homozygous haplotype associated with the disease in one of the affected brothers. The second brother died three months later because of septicemia and malignant hyperthermia. **Discussion and Conclusion:** The interest of the indirect diagnosis by genotyping is important to show the type of haplotype transmission among patients. This should be followed by sequencing the *NTRK1* gene looking for variations. This genetic study of this disease is important for prenatal diagnosis because there is no cure.

## P09.080

**Prevalence and clinical characterization of SPG31 in Spain**

A. Ordóñez-Ugalde<sup>1,2</sup>, J. Liñares-Blanco<sup>1,2</sup>, B. Quintáns<sup>1,3</sup>, P. Blanco-Arias<sup>1,4</sup>, M. Arias<sup>1,5</sup>, S. Piñeiro<sup>1</sup>, F. Grandas<sup>6</sup>, I. Sanz<sup>7</sup>, I. Pascual-Pascual<sup>7</sup>, A. López de Munain<sup>8</sup>, J. Arpa<sup>9</sup>, M. J. Sobrido<sup>1,4,3</sup>,

<sup>1</sup>Instituto de Investigacion Sanitaria, Santiago de Compostela, Spain, <sup>2</sup>USC, Santiago de Compostela, Spain, <sup>3</sup>CIBERER, Madrid, Spain, <sup>4</sup>Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain, <sup>5</sup>Complejo Hospitalario Universitario, Santiago de Compostela, Spain, <sup>6</sup>Hospital Universitario Gregorio Marañón, Madrid, Spain, <sup>7</sup>Hospital Universitario La Paz, Madrid, Spain, <sup>8</sup>Hospital Universitario Donostia, San Sebastián, Spain.

**BACKGROUND:** Hereditary spastic paraplegias (HSP) are genetically heterogeneous motor disorders, divided into pure (pyramidal syndrome) and complicated (additional manifestations). The prevalence and clinical spectrum of SPG31, one of the autosomal dominant HSPs, has not been studied in Spain.

**AIM:** To analyze frequency and phenotype of SPG31 in Spanish HSP patients.

**METHODS:** Through an NGS panel of HSP genes complemented by Sanger sequencing, we analyzed REEP1 in 104 index patients with HSP (51 pure, 50 complicated, 3 unknown) in whom SPAST (SPG4) and ATL1 (SPG3) point mutations had been excluded. Neurological examination and pedigree data were obtained. In order to establish pathogenicity of the variants we checked the literature and variant databases, performed *in silico* predictions and evaluated intrafamilial co-segregation.

**RESULTS:** Probably pathogenic variants in REEP1 were observed in 6 patients (11.7% among pure HSP without point mutations in SPAST and ATL1). Two were missense variants, two splicing variants, one nonsense, and a 9bp deletion involving REEP1 first three codons. Only one of these variants had been previously reported. We also identified a rare, silent variant of uncertain significance. All index cases had pure HSP, however cognitive and psychiatric manifestations were present in affected relatives. Onset age was very variable.

**CONCLUSIONS:** SPG31 represents ~12% of pure HSP patients in whom SPG4 and SPG3 have been ruled out by sequencing. This frequency is similar to other countries, and slightly lower than that of SPG3. The variable onset age and clinical spectrum difficult genotype-phenotype correlation and genetic counseling.

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## P09.081

**Epidemiological, Clinical, and genetic study in a large cohort of patients with spastic paraplegia**

L. Pedace<sup>1</sup>, C. Montecchiani<sup>1</sup>, A. Casella<sup>1</sup>, F. Gaudiello<sup>1</sup>, M. Miele<sup>1</sup>, R. Massa<sup>2</sup>, C. Caltagirone<sup>2,3</sup>, R. P. Munhoz<sup>4</sup>, J. L. Pedrosa<sup>5</sup>, O. G. P. Barrottini<sup>6</sup>, T. Kawarai<sup>6</sup>, A. Orlacchio<sup>1,7</sup>,

<sup>1</sup>Laboratorio di Neurogenetica, IRCCS Santa Lucia, Rome, Italy, <sup>2</sup>Medicina dei Sistemi, Università di "Tor Vergata", Rome, Italy, <sup>3</sup>Laboratorio di Neurologia Clinica e Comportamentale, IRCCS Santa Lucia, Rome, Italy, <sup>4</sup>Movement Disorders Centre, Toronto Western Hospital, University of Toronto, Toronto, ON, Canada, <sup>5</sup>Department of Neurology, Universidade Federal de São Paulo, São Paulo, Brazil, <sup>6</sup>Department of Clinical Neuroscience, University of Tokushima, Tokushima, Japan, <sup>7</sup>Dipartimento di Scienze Chirurgiche e Biomediche, Università di Perugia, Perugia, Italy.

**Introduction:** Mutations in the SPG4/SPAST gene are the major cause of hereditary spastic paraplegias (HSPs), a group of genetic disorders leading to

progressive spasticity and weakness of the lower limbs. This study includes the evaluation of a comprehensive spectrum of clinical features and the mutational screening of the *SPG4/SPAST* gene in patients with HSP worldwide. Patients and Methods: A large cohort of patients were recruited from Italian, Brazilian, and Japanese populations in a period from 2008 to 2016. Clinical and instrumental functional analyses consisted of neurological assessment and neuroimaging. Mutational screening was carried out by Sanger sequencing and MLPA analysis. Haplotype studies were also performed. Results: Our study highlights clinical and epidemiological differences among populations, showing unique genotype-phenotype correlations. Genetic analysis revealed a total of 52 different pathogenic changes in 284 patients: 21 sporadic cases and 263 from 96 families. Among them, six variants were novel and pathogenic. The analysis revealed a great portion of private mutations worldwide and confirmed the founder effect for one recurrent variant in the Italian population. Interestingly, mutations were detected in 21% of sporadic cases and in a range from 16% to 100% of families, depending on the number of affected in the family. Conclusion: This study represents the first worldwide *SPG4/SPAST* genetic screening on HSP patients. Epidemiological and clinical results broaden the spectrum of the clinical presentations associated with mutations in *SPG4/SPAST*. Finally, our findings provide evidence that the chance to detect *SPG4/SPAST* mutations varies proportionally to the number of affected in the family.

#### P09.082

#### Whole exome sequencing data analysis in recessive hereditary spastic paraplegia patients from Turkey

B. Ozes<sup>1</sup>, A. Candayan<sup>1</sup>, O. Ari<sup>1</sup>, Y. Parman<sup>2</sup>, E. Battaloglu<sup>1</sup>;

<sup>1</sup>Bogazici University, Istanbul, Turkey, <sup>2</sup>Istanbul University, Istanbul, Turkey.

Hereditary Spastic Paraplegia (HSP) is a group of clinically and genetically heterogeneous neurodegenerative disorders. Lower limb spasticity and progressive weakness are observed in 'pure' HSP patients. In 'Complicated' HSP, additional neurological and non-neurological symptoms are observed. HSP can be inherited in autosomal dominant, autosomal recessive, or X-linked manner. 49 loci and 43 genes are associated with autosomal recessive form of HSP (ARHSP). One patient from each of twenty ARHSP families from Turkey is selected for whole exome sequencing (WES). After the determination of candidate variations, segregation analyses were performed in the families to confirm that the variation might be responsible for the HSP phenotype. In three of the families variations were identified in known HSP genes and segregation with the disease in the families are confirmed for these variations. Two of them are identified in *SPG11* gene and they are c.1235C>G (p.Ser412Ter) variation in family P463 and c.6215\_6219dupAGAT (p.Phe2074ArgfsTer15) variation in family H16. The other variation confirmed is c.825T>A (p.Y275X) in *CYP7B1* gene in family H98. In eight of the families, various variations in HSP genes have been identified and in seven of the families, variations identified in the genes that are related to other neurological diseases. The segregation analyses are currently being performed for these families. For the remaining two families, WES data is still under analysis. With seven novel HSP gene candidates, our WES analysis gives promising preliminary data for identification of novel genes in families from Turkey and it suggests evidence for further genetic heterogeneity in ARHSP.

#### P09.083

#### Rapid and highly sensitive screen for HTT CAG repeat expansions in Huntington disease using a one-step triplet-primed PCR and melt curve analysis strategy

M. Zhao<sup>1</sup>, F. S. H. Cheah<sup>2</sup>, M. Chen<sup>1</sup>, C. G. Lee<sup>3,4,5</sup>, H. Law<sup>6</sup>, S. S. Chong<sup>1,2,7</sup>;

<sup>1</sup>Departments of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, <sup>2</sup>Khoo Teck Puat – National University Children's Medical Institute, National University Health System, Singapore, Singapore,

<sup>3</sup>Departments of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, <sup>4</sup>Division of Medical Sciences, National Cancer Center, Singapore, Singapore, <sup>5</sup>Duke-NUS Graduate Medical School, Singapore, Singapore,

<sup>6</sup>Department of Pediatric Medicine, KK Women's and Children's Hospital, Singapore, Singapore, <sup>7</sup>Department of Laboratory Medicine, National University Hospital, National University Health System, Singapore, Singapore.

Introduction: Molecular diagnosis of HD can be performed confidently using triplet primed PCR (TP-PCR) which detects all expanded alleles regardless of size. However, rapid high-throughput HD screening strategies currently rely on PCR using primers that flank the repeat, which may fail to detect large expansions and lead to false negative results. We describe an improved one-step screen for *HTT* expansion mutations based on MCA of TP-PCR products.

Materials and Methods: The assay was optimized on 30 genotype-known

cell-line DNAs, and two plasmids *pHTT(CAG)<sub>26</sub>* and *pHTT(CAG)<sub>33</sub>* were used to establish the threshold temperatures (TTs) distinguishing normal from expanded alleles. A blinded analysis was performed on 69 genotype-known clinical samples. Potential effects of reagent variations and common PCR contaminants were also evaluated.

Results: All 30 cell-line DNAs generated distinct melt peaks and *T<sub>m</sub>*'s which correlated well with each sample's larger allele. The TTs accurately distinguished between samples that carried normal-only, intermediate and expanded alleles. A companion protocol allowed rapid sizing confirmation of screen-positive samples, including one carrying a ~175-repeat expansion. Blinded analysis demonstrated clear segregation between unaffected and affected clinical samples (100% analytical sensitivity and specificity). The assay worked well with 10ng to 1ug of input DNA from blood, saliva or buccal swab. Sodium acetate contamination increased *T<sub>m</sub>*, whereas glycogen contamination had no effect.

Conclusions: Rapid, accurate, and high-throughput detection/exclusion of HD for differentiation from diseases with similar phenotypes can be achieved using this simple one-step screening assay, at one-third the cost of fluorescent PCR and capillary electrophoresis (€1.2/sample).

#### P09.084

#### Predictive testing on adult onset neurodegenerative diseases on social and personal life - a pilot study

P. E. Cohn-Hokke<sup>1</sup>, J. C. van Swieten<sup>1,2</sup>, A. Tibben<sup>3</sup>, E. J. Meijers-Heijboer<sup>1</sup>, J. A. Kievit<sup>2</sup>;

<sup>1</sup>VU University Medical Center, Amsterdam, Netherlands, <sup>2</sup>Erasmus Medical Center, Rotterdam, Netherlands, <sup>3</sup>Leiden University Medical Center, Leiden, Netherlands.

Background: Follow-up studies on predictive testing for neurodegenerative diseases mainly focussed on psychological outcome such as depression, anxiety and distress. Studies addressing the impact of predictive testing on employment, financial issues, lifestyle, relations and family life are scarce.

Objective: To investigate whether the course of life of mutation carriers of adult-onset neurodegenerative diseases differs negatively from non-carriers and untested at risk individuals.

Methods: Individuals, aged  $\geq 35$  years, tested while asymptomatic for Huntington's disease, frontotemporal dementia or Alzheimer's disease more than 2 years before the start of the study or at 50% risk for one of these diseases, were invited to complete a questionnaire of 70 items. Within a year, an additional questionnaire was completed, comprising 47 items with adjusted and additional questions. Of the 313 selected individuals, 17 carriers, 30 non-carriers and 27 untested persons responded, fulfilled the criteria and completed both questionnaires.

Results: We found no significant differences between carriers and non-carriers or untested individuals at risk in employment, financial situation and lifestyle or anxiety and depression. Carriers were more often single and childless, though these differences were not significant.

Conclusion: Although the low response rate requires caution when interpreting our observations, the findings of this pilot study suggest that the outcome of predictive testing on adult onset neurodegenerative diseases does not have a large negative effect on social and personal life.

#### P09.085

#### Mutations in the ABCC6 gene are associated with an increased risk for ischemic stroke

O. M. Vanakker<sup>1</sup>, E. Y. G. De Vilder<sup>1</sup>, S. Cardoen<sup>2</sup>, M. J. Hosen<sup>3</sup>, D. Hemelsoet<sup>2</sup>;

<sup>1</sup>Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Department of Neurology, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

Background. Evidence is emerging that ischemic stroke (IS) results from a complex interplay between environmental and genetic risk factors. As heterozygous ABCC6 mutations were shown to be associated with increased cardiovascular risk, we evaluated the role of ABCC6 in cryptogenic IS.

Methodology. Direct sequencing of ABCC6 was performed in 424 consecutive cryptogenic IS patients and 250 healthy age- and sex-matched controls. Allelic frequency differences were analyzed using a two-tailed Fisher's Exact test. Logistic regression analysis assessed modification of mutation-stroke interaction by cardiovascular risk factors. In one familial case with an autosomal dominant inheritance pattern of IS, segregation of the identified ABCC6 mutation was studied.

Results. Eighteen carriers of one ABCC6 mutation were identified compared to 2 carriers in controls (Odds Ratio 5.4975 [ $p = 0.023$ ; 95% CI 1.2-23.8]). No interaction with other CV risk factors was confirmed. In one family, we established segregation of an ABCC6 mutation in 18 family members with repetitive ischemic stroke and/or cardiovascular disease at young age and

simultaneously identified two novel PXE patients.

Conclusion. The segregation of an ABCC6 mutation in affected members of a multi-generation family with cerebrovascular disease suggests ABCC6 mutations to be significant risk factors for IS. This was confirmed by a high incidence of ABCC6 mutations in a cohort of cryptogenic IS patients compared to controls. As also demonstrated by the diagnosis of two novel PXE patients, identification of ABCC6 mutation carriers can have important implications for genetic counseling and follow-up of these families.

#### P09.086

##### A new phenocopy of Kabuki syndrome

*C. Lintas<sup>1</sup>, I. S. Piras<sup>2,3</sup>, M. Verdecchia<sup>4,1</sup>, C. Picinelli<sup>2</sup>, R. Sacco<sup>1</sup>, G. Merla<sup>5</sup>, M. Canali<sup>1</sup>, A. M. Persico<sup>1,3,6</sup>;*

<sup>1</sup>*University Campus Bio-Medico of Rome, Rome, Italy, <sup>2</sup>The Translational Genomics Research Institute, Phoenix, AZ, United States, <sup>3</sup>Mafalda Luce Center for Pervasive Developmental Disorders, Milan, Italy, <sup>4</sup>Azienda socio sanitaria G Mancinelli, Montelparo (FM), Italy, <sup>5</sup>IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy, <sup>6</sup>University of Messina, Messina, Italy.*

We report on a 14 year old man who had received a clinical diagnosis of Kabuki syndrome when he was six. No mutations in the two associated Kabuki genes, KMT2D and KDM6A, were found by direct sequencing and Multiple Ligation Probe Amplification. Array CGH revealed a de novo 5 Mb deletion on chromosome 10p11.22-11.21 encompassing 15 genes. Enrichment analysis by Gene Ontology showed that some of the deleted genes (as ZEB1, NRP1, FZD8, PARD3 and ITGB1) play an important role in neurodevelopment whereas others are implicated in chromatin remodeling. Particularly, the histone modifier EPC1 gene falls within the same "epigenetic functional classes" of the two candidate Kabuki syndrome genes and it is functionally linked to these by means of H2A histone. Consistent with this finding we demonstrated a significant reduction in EPC1 transcript which should functionally equate the loss of function mutations associated with the two Kabuki candidate genes.

#### P09.087

##### Recurrent mutation in the KCNC3 gene causes strikingly different clinical picture of SCA13 in a single family

*A. Todorova<sup>1,2</sup>, T. Todorov<sup>2</sup>, P. Palaima<sup>3</sup>, E. De Vriendt<sup>3</sup>, V. Bojinova<sup>4</sup>, M. Koleva<sup>4</sup>, T. Chamova<sup>5</sup>, V. Mitev<sup>1</sup>, A. Jordanova<sup>1</sup>;*

<sup>1</sup>*Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria, <sup>3</sup>University of Antwerp, Antwerp, Belgium, <sup>4</sup>MHATNP "St. Naum", Sofia, Bulgaria, <sup>5</sup>Clinic of Neurology, University Hospital "Alexandrovska", Sofia, Bulgaria.*

We report a family with autosomal dominant spinocerebellar ataxia affecting two sibs and their mother. The clinical onset of the mother was around the age of 18-19 years with scanning speech and gait ataxia. Brain MRI demonstrated cerebellar atrophy at the age of 23 and she has been clinically diagnosed as spinocerebellar ataxia (SCA). The clinical onset of the sons was much earlier at the age of 2 and 1 years respectively with gait instability. The motor development of the second boy was delayed and independent inassisted gait has not been achieved since the age of 2.5 years. Neuropsychological development in both children was normal. The neurological examination revealed ataxia of stance and gait, intention tremor, scanning speech. Convergent strabismus was present in the elder brother. Their brain MRI at the age of 2 showed cerebellar hypotrophy.

The following dominant spinocerebellar ataxias SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, DRPLA were excluded. The genes TTBK2 for SCA11 and PRKCG for SCA14 were tested negative by Sanger sequencing. The NGS (WES) revealed the mutation c.1268G>A, p.Arg423His in the KCNC3 gene for SCA13. The mutation was confirmed by Sanger sequencing, it segregated with the disease in the family.

SCA13 accounts for less than 1% of SCAs. This mutation has been previously published. Typically it is associated with early onset, but in our family the mother showed weaker and late onset manifestation in comparison to both sons. SCA13 shows wide spectrum of phenotypes even in the frame of a single family.

#### P09.088

##### De novo KCNH1 mutations in four patients with syndromic developmental delay, hypotonia and seizures

*N. Matsumoto, R. Fukui, N. Miyake;*

*Yokohama City University Graduate School of Medicine, Yokohama, Japan.*

The voltage-gated Kv10.1 potassium channel, also known as ether-a-go-go-related gene 1, encoded by KCNH1 (potassium voltage-gated channel, subfamily H [eag-related], member 1) is predominantly expressed in the central

nervous system. Recently, de novo missense KCNH1 mutations have been identified in six patients with Zimmermann-Laband syndrome and in four patients with Temple-Baraitser syndrome. These syndromes were historically considered distinct. Here, we report three de novo missense KCNH1 mutations in four patients with syndromic developmental delay and epilepsy. Two novel KCNH1 mutations (p.R357Q and p.R357P), found in three patients, were located at the evolutionarily highly conserved arginine in the channel voltage sensor domain (S4). Another mutation (p.G496E) was found in the channel pore domain (S6) helix, which acts as a hinge in activation gating and mainly conducts non-inactivating outward potassium current. A previously reported p.G496R mutation was shown to produce no voltage-dependent outward current in CHO cells, suggesting that p.G496E may also disrupt the proper function of the Kv channel pore. Our report confirms that KCNH1 mutations are associated with syndromic neurodevelopmental disorder, and also support the functional importance of the S4 domain.

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#### P09.089

##### Phenotypic characterisation of a recurrent homozygous deleterious variant in the KCTD3 gene

*E. A. Fageih<sup>1</sup>, A. A. Alfares<sup>2</sup>, M. M. Saleh<sup>1</sup>;*

<sup>1</sup>*King Fahad Medical City, Riyadh, Saudi Arabia, <sup>2</sup>Department of Pediatrics, College of Medicine, Qassim University, Riyadh, Saudi Arabia.*

Introduction: An association between KCTD3 and multiple congenital anomalies has previously been published; here, we expand the phenotype associated with deleterious variants in KCTD3.

Case series: Six patients (one monozygotic twin) from three consanguineous families presented with early-onset severe psychomotor retardation (100%), epilepsy (100%), hypotonia (100%), spasticity (33%), and dysmorphic features (33%). Brain MRI revealed Dandy-Walker malformation (84%) and hydrocephalus (33%). Abdominal ultrasound revealed unilateral multicystic kidney disease (33%). Basic metabolic, biochemical, haematological work up and aCGH were unremarkable. Whole exome sequencing identified previously described homozygous likely pathogenic variant in KCTD3 c.166C>T (p. (Arg56\*) by the same research group in a one-year-old patient with a similar presentation.

Discussion: KCTD3 belongs to a family of accessory subunits that regulate the biophysical properties of ion channels. The disruption of this gene has been associated with autism and renal aging in mice. It is highly expressed in the kidney and brain. Complete loss of this gene in the six patients resulted in specific clinical phenotypes mainly affecting brain and kidney development.

Conclusion: We report on six patients with recurrent deleterious variants in KCTD3. To the best of our knowledge, this is the largest case series of a similar presentation and provides additional observations regarding the role of KCTD3 in brain and kidney development. The universal involvement of cerebellum in those patients with the KCTD3 homozygous mutation make it a new member to the expanding lists of cerebellar hypoplasia-associated genes. However, its exact neurocerebellar role, function and phenotypic effects need extensive functional study.

#### P09.090

##### A de novo KIF1A mutation in a patient having epileptic encephalopathy with PEHO-like features

*A. Laari<sup>1,2,3</sup>, M. Muona<sup>1,3,4</sup>, C. Courage<sup>1,2,3</sup>, M. Somer<sup>5</sup>, A. Lehesjoki<sup>1,2,3</sup>, A. Anttonen<sup>1,6,7</sup>;*

<sup>1</sup>*Folkhalsan Institute of Genetics, Helsinki, Finland, <sup>2</sup>Neuroscience Center, University of Helsinki, Helsinki, Finland, <sup>3</sup>Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland, <sup>4</sup>Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland, <sup>5</sup>Norja Centre, Rinnekoti Foundation, Helsinki, Helsinki, Finland, <sup>6</sup>Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland, <sup>7</sup>Laboratory of Genetics, Helsinki University Hospital, Helsinki, Finland.*

PEHO syndrome (Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy; MIM 260565) is an autosomal recessively inherited progressive infantile encephalopathy. A significant number of patients presents with many of the clinical features of PEHO syndrome but do not show typical neuroradiological findings or progression of cerebellar atrophy and are classified as having PEHO-like syndrome. We recently identified the recessive founder mutation responsible for PEHO syndrome in Finnish patients (Anttonen et al, in revision). Langlois et al. (Eur J Hum Genet 2015) reported that PEHO syndrome is caused by de novo dominant variants in the motor domain of the KIF1A gene. In our exome study of 33 PEHO-like patients negative for the PEHO founder mutation we identified one patient with a de novo missense variant (c.757G>A;p.E253K) affecting the motor

domain of KIF1A. The variant has been published earlier (Lee et al., Human Mutation, 2014) in two patients with intellectual disability, cerebellar and optic atrophy and axonal neuropathy. Our patient presented with progressive cerebellar atrophy, optic atrophy and dysmorphic features quite similar to PEHO syndrome. In thorough evaluation of the imaging findings in different time points, the dysmyelination was more severe in this patient than in typical PEHO patients. Thus, the patient was concluded to have PEHO-like features.

Our findings, combined with analysis of data on patients reported in the literature to have likely pathogenic variants affecting the motor domain coding region of KIF1A imply that de novo mutations in KIF1A are associated with a PEHO-like phenotype rather than PEHO-syndrome.

## P09.091

### Kleefstra syndrome and infantile spasms: a rare association

*S. Dimassi<sup>1</sup>, N. Soyah<sup>2</sup>, A. Labalme<sup>3</sup>, D. Sanlaville<sup>2</sup>, L. Boughamoura<sup>2</sup>, A. Saad<sup>1</sup>, S. Mougou-Zerelli<sup>1</sup>;*

<sup>1</sup>*Department of Cytogenetics and Reproductive Biology, Farhat Hached University Teaching Hospital, Sousse, Tunisia, <sup>2</sup>Department of Pediatrics, Farhat Hached University Teaching Hospital, Sousse, Tunisia, <sup>3</sup>Department of Genetics, Lyon University Hospital, Lyon, France.*

Kleefstra syndrome is characterized by moderate to severe intellectual disability (ID), childhood hypotonia and distinct facial features. Additional findings can also be observed including heart defects, renal/urologic defects, genital defects in males, severe respiratory infections, autistic-like features in childhood, epilepsy,... The syndrome can be caused either by a submicroscopic 9q34.3 deletion or an intragenic haploinsufficiency mutation of the euchromatin histone methyltransferase 1 (EHMT1) gene.

We report here, the first 3-years old boy of non consanguineous parents, with severe ID, hypotonia, facial dysmorphism, cardiac defect, severe respiratory infection and infantile spasms (IS). Standard Karyotype was normal and array chromosome genomic hybridization (array CGH) showed a 826 kb 9q34.3 deletion ranging from 139,970,775 to 140,796,632 pb (hg19). The 9q de novo telomeric deletion was confirmed by FISH using 9q telomeric probe.

Epilepsy and Seizures of various types occur in 25 to 35% of patients. Only two cases of kleefstra syndrome associated to IS have been published to date. We described the third case of epileptic IS, an age related seizure type, occurring usually in clusters and characterized by a typical electrodecremental pattern and various interictal electroencephalographic abnormalities. EHMT1, encoding a histone H3 Lys 9 methyltransferase and involving in chromatin remodeling, accounts for the majority of features in Kleefstra syndrome and is probably responsible of the epilepsy phenotype.

## P09.092

### Novel mutation in POLR1C in RNA polymerase III-related leukodystrophy with myoclonus

*A. Karkar<sup>1</sup>, I. Kraoua<sup>2</sup>, C. Drissi<sup>2</sup>, S. Samaan<sup>2</sup>, F. Renaldo<sup>3</sup>, M. Elmaleh<sup>3</sup>, I. Dorboz<sup>4</sup>, O. Boespflug-Tanguy<sup>2</sup>;*

<sup>1</sup>*Genetics and Molecular Pathology Laboratory, Casablanca, Morocco, <sup>2</sup>National Institute Mongi Ben Hamida of Neurology, Tunis, Tunisia, <sup>3</sup>Robert Debré Hospital, Paris, France, <sup>4</sup>INSERM, Paris, France.*

RNA polymerase III (POLR3)-related leukodystrophy is an autosomal recessive neurodegenerative disorder characterized by childhood onset of progressive motor decline manifest as spasticity, ataxia, tremor, and cerebellar signs, as well as mild cognitive regression. Other features may include hypo/oligodontia and hypogonadotropic hypogonadism. This disorder is caused by mutations in *POLR3A* and *POLR3B* gene. Recently Thiffault et al. have identified a new gene *POLR1C* related to this disorder. This gene is also mutated in some Treacher Collins syndrome cases. Here we describe a novel mutation in a 23 year-old girl from a consanguineous Tunisian family. At the age of 5 years, she developed unsteady gait, hand tremor, dysarthria and movement disorders. The first examination at 13 years of age showed cerebellar syndrome, pyramidal signs in upper and lower limbs and generalized myoclonus. Brain MRI showed diffuse hyperintense signal of the supratentorial and cerebellar white matter, cerebellar atrophy and hypointensity on the T2-weighted images of the ventrolateral thalamus. The corpus callosum is slightly thinned. She was negative for NGS leukodystrophy-panel containing twenty six most frequently involved genes including the *POLR3A* and *POLR3B* genes used by the routine molecular diagnostic lab of Robert Debré Hospital. Sanger sequencing of the coding regions of *POLR1C* gene revealed a homozygous mutation (c.863T>C/ p.Phe288Ser). This mutation is a novel *POLR1C* mutation. This result confirms that the analysis of *POLR1C* gene is an additional way for POLR3-related leukodystrophy diagnosis in which severe non epileptic myoclonus and T2 hypointensity of the thalamus were not reported.

## P09.093

### Combined genetic and neuroradiological assessment of neuronal migration disorders

*U. Hehr<sup>1</sup>, S. Hinreiner<sup>1</sup>, T. Geis<sup>2</sup>, G. Schuierer<sup>3</sup>, S. M. Herbst<sup>1</sup>;*

<sup>1</sup>*Center for and Department of Human Genetics, University Regensburg, Regensburg, Germany, <sup>2</sup>KUNO Children's University Hospital Regensburg, Regensburg, Germany,*

<sup>3</sup>*Division of Neuroradiology, University Hospital Regensburg, Regensburg, Germany.*

**Introduction:** Neuronal migration disorders (NMD) are a clinically and genetically important cause of early developmental delay, cognitive impairment and seizures. A substantial proportion of practically all morphological forms has been associated with a wide variety of isolated or syndromal monogenic disorders.

**Method:** Conventional Sanger sequencing and CNV analysis by MLPA, more recently massive parallel NGS gene panel sequencing (MGPS), additional neuroradiological assessment of available cerebral MR (cMR) imaging.

**Results:** Conventional Sanger sequencing with MLPA in our laboratory allowed a genetic classification (mutation class VUS4 or VUS5) for 21.6% of our overall NMD patient cohort (981 independent patients with lissencephaly/pachygryria/polymicrogyria, double cortex, periventricular nodular heterotopia or complex NMD; 1.53 genes per patient). For a subgroup of 146 patients with cMR imaging available for pretest reevaluation causal mutations were identified in 30.8%.

Implementation of an individual combined diagnostic strategy including massive parallel NGS gene panel sequencing MGPS (45.8 genes per patient) and neuroradiological assessment of patients with NMD now allows not only a substantially faster and more cost-effective workup, but also further increases the diagnostic yield through detection of mutations in genes with small contribution (e.g. DYNC1H1 or TREX1) and by screening for copy number variations (e.g. LIS1) for all tested genes within the known current technical limitations. Furthermore, sufficient MGPS coverage also increases the sensitivity to detect somatic mosaicism (e.g. DCX).

**Summary:** Our preliminary findings indicate a benefit of diagnostic MGPS for NMD, when combined with critical data assessment and interpretation in the context of individual clinical and neuroradiological findings.

## P09.095

### Genetic studies of multiple consanguineous families with primary microcephaly revealed pathogenic variants in ASPM and CDK6

*M. A. Khan<sup>1,2</sup>, M. Z. Ali<sup>2</sup>, M. Kuster<sup>3</sup>, N. Ahmad<sup>4</sup>, V. Rupp<sup>3</sup>, H. Gul<sup>2</sup>, S. Abbas<sup>2</sup>, V. Kumar<sup>1</sup>, F. A. Mir<sup>1</sup>, S. Khan<sup>1</sup>, R. M. Mohammad<sup>1</sup>, C. Windpassinger<sup>3</sup>, Z. Nawaz<sup>2</sup>;*

<sup>1</sup>*Hamad Medical Corporation, Doha, Qatar, <sup>2</sup>Gomal University, D.I.Khan, Pakistan,*

<sup>3</sup>*Medical University of Graz, Graz, Austria, <sup>4</sup>Hazara University, Mansehra, Pakistan.*

**Background:** Primary microcephaly is a rare genetic disorder that is characterized by reduced head circumference and intellectual disability. Genetic studies have identified 15 (MCPH) causative genes which include MCPH1, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1, CDK6, CENPE, SASS6 and MFSD2A. Physiologically, most of the protein products of these MCPH genes are involved in cell cycle and its regulation.

**Methods:** The genomic analysis was performed through whole exome sequencing, while co-segregation of the identified pathogenic variants in the corresponding family members was carried out by sanger DNA sequencing. **Results:** In the present genetic study, we present three consanguineous Pakistani families segregating primary microcephaly that are ascertained from the rural area of Khyber-Pukhtunkhwa province of Pakistan. Sequence analysis of two families revealed one novel [NM\_018136.4: c.10013delA (p.Asp338Valfs\*2)] and a previously reported [NM\_018136.4: c.9730C>T (p.Arg3244\*)] mutation in ASPM gene. The novel frame-shift mutation (p.Asp338Valfs\*2) in ASPM presumably truncate the protein synthesis that result in loss of armadillo type fold domain. The third family showed the same missense mutation [NM\_001259.6: c.589G>A (p. Ala197Thr)] in CDK6 gene as recently identified in a single Pakistani family.

**Conclusion:** ASPM is the most prevalent gene for MCPH in south Asian populations, and our study also expanded its mutational spectrum. The reported CDK6 gene has previously been mapped in a consanguineous Pakistani family, and here we are reporting the second family. These results further extend the evidence of CDK6 gene implication in MCPH and suggest its founder effect in the Pakistani population.

## P09.096

### 2002-2016: What is known about reported MCPH patients?

P. Létard<sup>1,2,3</sup>, P. Gressens<sup>4,5</sup>, A. Verloes<sup>5,6</sup>, S. Passemard<sup>1,5,6</sup>;

<sup>1</sup>INSERM U1141 PROTECT, Paris, France, <sup>2</sup>Service d'anatomie et cytologie pathologiques - CHU Jean Verdier, Bondy, France, <sup>3</sup>Université Paris 13, Sorbonne Paris Cité, Bobigny, France, <sup>4</sup>Center for developing brain, King's college, St. Thomas' Campus, London, United Kingdom, <sup>5</sup>Université Paris Diderot, Sorbonne Paris Cité, Paris, France, <sup>6</sup>Département de génétique, Hôpital Robert Debré, Paris, France.

**Introduction:** Microcephaly primary hereditary (MCPH) is a rare autosomal recessive neurodevelopmental disorder characterized by a reduction in brain growth, associated with an intellectual disability. MCPH is genetically heterogeneous with fifteen genes identified (MCPH1-15), coding proteins related to the centrosome. Here, we review clinical, molecular and radiological findings of reported MCPH patients and highlight prognostic factors.

**Materials and methods:** We have performed an online literature research in PubMed database to identify any patient with mutations in MCPH genes. **Results:** 722 patients (326 families) have been reported, with a high rate of consanguinity, 44% are of Pakistani origin. In Europe, there are 41 families, often not-related. Mutations in ASPM represent the most frequent cause of MCPH (63% of families), followed by mutations in WDR62 (14%) and MCPH1 (6%) genes. 234 MCPH mutations have been reported, with a majority of nonsense and frameshift mutations. Intellectual disability is mild to moderate but neuropsychological assessment was almost never carried out (4% of reported patients). When performed, CDK5RAP2 patients have a better IQ than ASPM patients. A brain MRI was performed in 16% of patients: it was abnormal in 84%, and frequent abnormalities include: gyral simplification, corpus callosum abnormality and pachygryria.

**Conclusion:** 722 patients have been reported with mutations in one of the 15 MCPH genes but clinical, neuropsychological and radiological data are often lacking, which makes genotype-phenotype correlations or genetic counseling still difficult. How these mutations alter brain structure and cognitive functions are major questions that we have now to solve in MCPH field.

## P09.097

### MECP2 duplication: genetic and clinical study in Spanish patients

L. Blasco Pérez<sup>1</sup>, S. Vidal Falcó<sup>1</sup>, E. Gean Molins<sup>2</sup>, N. Brandi Tarrau<sup>2</sup>, P. Pacheco Fernandez<sup>2</sup>, M. del Campo<sup>3</sup>, V. Catalá<sup>4</sup>, E. Guillén<sup>5</sup>, P. Lapunzina<sup>6</sup>, E. Lloveras<sup>7</sup>, H. Maortua<sup>8</sup>, F. Martínez<sup>9</sup>, A. Mesas<sup>10</sup>, J. Nevado<sup>6</sup>, M. Obón<sup>11</sup>, C. Orellana<sup>9</sup>, A. Plaja<sup>12</sup>, M. Rosello<sup>9</sup>, M. I. Tejada<sup>9</sup>, E. Tizzano<sup>12</sup>, J. Armstrong Morón<sup>13</sup>;

<sup>1</sup>Fundació Sant Joan de Deu, Barcelona, Spain, <sup>2</sup>Hospital Universitario San Juan de Dios, Barcelona, Spain, <sup>3</sup>Hospital Valle Hebrón, Barcelona, Spain, <sup>4</sup>Universidad Autónoma de Barcelona, Barcelona, Spain, <sup>5</sup>Hospital Virgen de la Arrixaca, Murcia, Spain, <sup>6</sup>Instituto de Investigación Sanitaria del Hospital Universitario La Paz, Madrid, Spain, <sup>7</sup>LABCO-Iberia, Barcelona, Spain, <sup>8</sup>Hospital Universitario de Cruces, Bilbao, Spain, <sup>9</sup>Hospital Universitario y Politécnico La Fe, Valencia, Spain, <sup>10</sup>Hospital Xanit, Málaga, Spain, <sup>11</sup>Laboratoris ICS, Girona, Spain, <sup>12</sup>Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>13</sup>Hospital Sant Joan de Deu, Barcelona, Spain.

**Introduction:** The MECP2 duplication syndrome (OMIM\_300260) is a neurodevelopmental disorder X-linked characterized by severe to profound intellectual disability, early infantile hypotonia, autistic traits, seizures and recurrent respiratory infections. It usually affects boys, but also there are girls affected. Duplication could be de novo or inherited from asymptomatic carrier mother. It has been reported about 120 cases all over the world, without a known incidence.

The aim of the study is to characterize a Spanish cohort with MECP2 duplication syndrome to improve our knowledge of the disease and perform a genotype-phenotype correlation.

**Material and Method:** The cohort consists in 13 patients of both sexes diagnosed in different Spanish hospitals. The duplications were detected by MLPA and/or CGH-array. The clinical characterization was carried out using a checklist designed for the project. The molecular characterization is divided into several steps: 1) Checking the duplication by qPCR-doses, study XCI and FISH; 2) If FISH shows tandem duplication, we narrowed the breakpoints through qPCR, PCR-long and Sanger sequencing; 3) Analyze the expression of the two MeCP2 isoforms by RT-qPCR.

**Results:** In this collaborative study has been characterized a heterogeneous cohort, with both sexes, with different phenotypes (Rett-like and duplication patients) and inherited or de novo duplication located in different regions (tandem, other region of ChrX or ChrY). However, this information is not enough to create a clear genotype-phenotype correlation.

**Conclusions:** We suppose MECP2 duplication syndrome is an underdiagnosed disease that needs further characterization studies in order to give a better genetic and clinical diagnosis.

## P09.098

### Exploring Allele Specific Methylation (ASM) in drug dependence susceptibility

L. Pineda-Cirera<sup>1,2,3</sup>, J. Cabana-Domínguez<sup>1,2,3</sup>, C. Roncero<sup>4,5,6</sup>, L. Rodríguez-Cintas<sup>4,5</sup>, L. Grau-López<sup>1,5,6</sup>, N. Martínez-Luna<sup>4,5</sup>, A. C. Abad<sup>4,5</sup>, M. Sorribes<sup>4,5</sup>, M. Casas<sup>5,6,7</sup>, J. A. Ramos-Quiroga<sup>5,6,7</sup>, M. Ribasés<sup>5,6,8</sup>, B. Cormand<sup>1,2,3</sup>, N. Fernández-Castillo<sup>1,2,3</sup>;

<sup>1</sup>Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain,

<sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain, <sup>4</sup>Addiction and Dual Diagnosis Unit, Psychiatric Services, Hospital Universitari Vall d'Hebron-ASPB, Barcelona, Spain, <sup>5</sup>Biomedical Network Research Center on Mental Health (CIBERSAM), Instituto de Salud Carlos III, Barcelona, Spain,

<sup>6</sup>Department of Psychiatry, Hospital Universitari Vall d'Hebron, Barcelona, Spain,

<sup>7</sup>Department of Psychiatry, Universitat Autònoma de Barcelona, Barcelona, Spain,

<sup>8</sup>Psychiatric Genetics Unit, Hospital Universitari Vall d'Hebron, Barcelona, Spain, Barcelona, Spain.

**Introduction:** Drug addiction is a neuropsychiatric disorder in which both environmental and genetic factors are involved. Work over the past decade has demonstrated a crucial role of epigenetic mechanisms in driving long-lasting changes in gene expression in several tissues, including the brain.

**Material and methods:** Starting from a previously reported list of 2,878 SNPs that correlate with differential levels of methylation at CpG sites (allele-specific methylation), we obtained a sub-list of 88 SNPs using the following selection criteria:  $R^2 > 0.5$ , only one SNP for each CpG site. Subsequently, we evaluated the possible contribution to drug dependence predisposition of these SNPs in a sample of 697 drug-dependent patients and 656 sex-matched controls from Spain and in a replication sample.

**Results:** A single SNP, rs3766612, was found associated with drug addiction ( $p\text{-value}=0.00071$ ;  $\text{OR-95\%CI}=1.41$  (1.16-1.69)) and survived the Bonferroni correction for multiple testing. We then aimed at replicating this finding in a follow-up sample of 740 drug-dependent patients and 771 sex-matched controls, but rs3766612 was not found associated with drug addiction. Finally, we performed a pooled analysis including the discovery and replication samples where the SNP rs3766612 remained associated with drug addiction in the combined dataset ( $p\text{-value}=0.00655$ ;  $\text{OR-95\%CI}=1.19$  (1.05-1.35)).

**Conclusion:** We detected a potential association between drug addiction and SNP rs3766612, which correlates with differential levels of methylation at the CpG island cg23166289. Interestingly, this CpG site is located in a genomic region that contains several genes belonging to families previously related to drug dependence, such as SYT14, LAMB3 or CAMK1G.

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## P09.099

### MSTO1 is a cytoplasmic protein involved in mitochondrial fusion dynamics and its human mutation is associated with mitochondrial myopathy

A. Gal<sup>1,2</sup>, P. Balicza<sup>1</sup>, D. Weaver<sup>2</sup>, S. Naghdi<sup>2</sup>, J. K. Suresh<sup>2</sup>, G. Inczedy-Farkas<sup>1</sup>, V. Reményi<sup>1</sup>, P. Várnai<sup>2</sup>, T. Gyuris<sup>2</sup>, A. Farkas<sup>2</sup>, L. Nagy<sup>2</sup>, M. J. Molnar<sup>1</sup>, G. Hajnóczky<sup>2</sup>;

<sup>1</sup>Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest, Hungary, <sup>2</sup>MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA, United States, <sup>3</sup>Department of Physiology, Semmelweis University, Budapest, Hungary, <sup>4</sup>Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary.

**Introduction:** MSTO1 protein has been localized to mitochondria and linked to mitochondrial morphology but its specific role remained unclear. In this study, we investigated the role of MSTO1 on mitochondrial dynamics and bioenergetics in patient-derived fibroblasts and HeLa cells.

**Methods:** The whole exome-sequencing was performed a Hungarian family with mitochondrial disease resulting in myopathy, hypoacusis and psychiatric symptoms. The mitochondrial fusion-fission and bioenergetics in both patient-derived cells and cell lines using genetic rescue and gene silencing were investigated by live cell imaging, fluorometric measurements and immunoblotting.

**Results:** In this study, we identified a c.22 G>A, (p.Val8Met) mutation of MSTO1 in our patients. In the patient fibroblasts, the MSTO1 mRNA and protein abundance is decreased, mitochondria show fragmentation, aggregation, decreased network continuity and fusion activity. These can be reversed by genetic rescue. Short term silencing of MSTO1 in HeLa cells reproduced the impairment of mitochondrial morphology and dynamics observed in the patient fibroblasts without impairing bioenergetics. Contrary to a previous report, MSTO1 is mainly localized in the cytoplasmic area and shows only partial colocalization with the mitochondria. After plasma membrane permeabilization, MSTO1 is released to the cytoplasm.

**Conclusions:** Our findings indicate that MSTO1 has a role in mitochondrial morphogenesis and quality control by supporting mitochondrial fusion. MSTO1 likely interacts with the mitochondrial fusion machinery as a solub-

le factor at the cytoplasm-mitochondrial outer membrane interface. Loss-of-function mutation in MSTO1 is could be associated with mitochondrial disorders.

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## P09.100 MLC1 mutational analysis in Korean patients with megalencephalic leukoencephalopathy with subcortical cysts

**B. Lim, H. Cheong;**  
Seoul National University Children's Hospital, Seoul, Korea, Republic of

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare inherited disorder characterized by macrocephaly, slow neurologic deterioration and seizures. Brain MRI shows extensive white matter signal abnormalities with subcortical cysts in the anterior temporal regions. The first causative gene, MLC1 is mutated in approximately 75% of patients and inherited as autosomal recessive trait. The defect in MLC1 has been known to hamper the ion and water homeostasis and osmotic balance in astrocytes, resulting chronic brain white matter edema. In this study, we present the clinical manifestation, neuroimaging and mutational analysis in the MLC patients first to be described from Korea. All of the four MLC patients had early onset macrocephaly, delay in the motor skills with a gradual onset of motor deterioration. Brain MRI showed typical MLC features. Two types of mutations were identified. p.A275D mutation was most commonly observed, six of eight (75%) alleles in Korean patients with MLC. Homozygous p.I113Gfs\*4 frameshift mutation was identified in only one patient while patient's mother was heterozygous state. By MLPA and CGH + SNP microarray analysis, segmental loss of heterozygosity of chromosome 22 including MLC1 region was confirmed indicating maternal uniparental disomy.

## P09.101 Sequencing in patients with familial and sporadic multiple sclerosis reveals the possible etiological role of rare and highly penetrant genetic variation

**A. Maver<sup>1</sup>, A. Hodzic<sup>1</sup>, S. Šega<sup>2</sup>, J. Drulović<sup>3</sup>, I. Novaković<sup>4</sup>, S. Ristić<sup>5</sup>, M. Kapović<sup>6</sup>, T. Pekmezović<sup>3</sup>, B. Peterlin<sup>1</sup>;**

<sup>1</sup>Clinical Institute of Medical Genetics, Ljubljana, Slovenia, <sup>2</sup>Department of Neurology, UMC Ljubljana, Ljubljana, Slovenia, <sup>3</sup>Neurology Clinic, CCS, Belgrade, Serbia, <sup>4</sup>Institute of Human Genetics, MF Beograd, Belgrade, Serbia, <sup>5</sup>Department of Biology and Medical Genetics, School of Medicine Rijeka, Rijeka, Croatia.

Multiple sclerosis (MS) is a chronic autoimmune neurological disorder with complex genetic architecture. Despite efforts, even large genome-wide association studies (GWAS) failed to completely explain heritability of MS based on common genetic variation.

To characterize the possible role of rare, highly penetrant genetic variants in MS, we performed whole exome sequencing in 48 patients with familial MS, 40 patients with sporadic MS and 92 population-matched controls. We have focused the analysis to rare (<5%) and predicted pathogenic variants (truncating or predicted pathogenic missense variants) in 102 genes associated with MS in the most recent GWAS performed to date.

In the subset of patients with familial MS, we identified two novel truncating variants of high impact in MMEL1 and ALPK2 genes. Truncating variants in two further GWAS genes - IL7R and AHI1 were also identified in sporadic MS cases. Several predicted pathogenic missense variants were also identified in MS associated genes: 18 variants in familial MS cases and 27 in sporadic cases. Finally, we could also demonstrate an increased burden of rare predicted pathogenic variants in GWAS genes in both familial (OR=1.77, p=0.048) and sporadic MS cases (OR=2.5, p=0.0003), when comparing this load to the population of healthy controls.

Our results suggest the possible role of rare pathogenic variants in genes previously associated with MS in association studies. Furthermore, we report an increased overall burden of pathogenic variants within these genes in familial and sporadic cases with MS.

## P09.102 Effect of genes in iron metabolism on multiple sclerosis development

**N. Starcevic Cizmarevic<sup>1</sup>, L. Lovrecic<sup>2</sup>, B. Curko-Cofek<sup>3</sup>, V. Barac-Latas<sup>3</sup>, M. Kapovic<sup>1</sup>, B. Peterlin<sup>2</sup>, S. Ristic<sup>1</sup>;**

<sup>1</sup>Department of Biology and Medical Genetics, Faculty of Medicine, Rijeka, Croatia,

<sup>2</sup>Clinical Institute of Medical Genetics, University Medical Centre, Ljubljana, Slovenia,

<sup>3</sup>Department of Physiology, Immunology and Pathophysiology, Faculty of Medicine, Rijeka, Croatia.

Introduction: Increased local iron concentration in brain parenchyma of multiple sclerosis (MS) patients is documented by magnetic resonance imaging, but role of iron in MS etiopathogenesis is still debated. Brain iron homeostasis is regulated by different factors, among which the transferrin and

hemochromatosis proteins seem to play a key role. The aim of study was to investigate whether HFE (C282Y, H63D), TF (P570S) and transferrin receptor (TFRC-S142G) gene variants contribute to MS development.

Materials and Methods: We genotyped 455 patients diagnosed with MS according to the revised McDonald criteria and 400 healthy controls from Croatia and Slovenia by PCR-RFLP or Real-time PCR method.

Results: A significantly higher frequency of the C282Y carrier mutation was observed in MS patients (6.3%) than in controls (3.1%) (P=0.033). Allele and genotypes frequencies for other polymorphisms did not differ significantly (P>0.05). A three year earlier onset was found in carriers of the C282Y mutation (P=0.106), and significantly earlier onset in TF-C2 homozygotes (P=0.016). Disease began eight years later in H63D homozygotes but statistical difference show borderline significance (P=0.056). Progression index of MS was higher in carrier of TFRC-S142G A allele also with borderline significance (P=0.051).

Conclusions: Our results indicate that HFE-C282Y mutation may be risk factor to MS susceptibility. Variants C282Y and TF-C2 are possible predictors for early onset of MS, while HFE-H63D prolong disease onset. Polymorphism TFRC-S142G might have influence on disease progression. Polymorphisms in HFE, TFRC and TF genes coding for iron binding and transporting proteins might contribute to pathogenesis of MS.

## P09.103 Genetic basis of neurodegeneration with brain iron accumulation in Spanish population

**C. Tello<sup>1</sup>, V. Lupo<sup>1</sup>, A. Darling<sup>2</sup>, B. Pérez-Dueñas<sup>2</sup>, C. Espinós<sup>1</sup>;**

<sup>1</sup>Centro de Investigación Príncipe Felipe, Valencia, Spain, <sup>2</sup>Hospital Sant Joan de Déu, Barcelona, Spain.

Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group of inherited neurologic disorders characterized by progressive movement disorders and abnormal accumulation of iron in brain, specifically in the basal ganglia. Mostly affected are children in whom the progress of the disease is devastating and includes progressive slurred speech, dysphagia, dystonia, spasticity, parkinsonism, pure akinesia, oculomotor dysfunction, loss of vision and neuropsychiatric symptoms. There are 10 genes causing NBIA that resolve 80% of cases; the remaining patients are cataloged as idiopathic cases. The NBIA-causing genes play a role in multiple biological processes such as iron metabolism, mitochondrial dynamics, reactive oxygen species (ROS)-induced damage, lipid metabolism and autophagy. Our aim is the genetic characterization of patients with NBIA in Spanish population.

To establish the genetic basis of these patients we have analyzed the most common genes (*PANK2*, *PLA2G6*, *WDR45* and *C19orf12*) responsible for the disease by Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) in a clinical series of 57 patients diagnosed with NBIA. To date we have achieved the molecular diagnosis for 40 NBIA patients: 31 patients carry mutations in *PANK2*, and 9 patients in *PLA2G6*. Eleven of these mutations are novel and functional studies are in progress with the aim to demonstrate its pathogenicity. No mutations were identified in the *C19orf12* or *WDR45* genes. We currently study the remaining six NBIA genes in the patients who remain undiagnosed by targeted next generation sequencing.

## P09.104 Copy number variants identification and clinical correlation in an adult population with intellectual disability and psychiatric/behavioural disorders

**M. Viñas-Jornet<sup>1</sup>, S. Esteba-Castillo<sup>2</sup>, N. Baena<sup>1</sup>, N. Ribas-Vida<sup>2</sup>, A. Ruiz<sup>2</sup>, D. Torrents-Rodas<sup>2</sup>, E. Gabau<sup>3</sup>, C. Aguilera<sup>1</sup>, R. Novell<sup>2</sup>, M. Guitart<sup>1</sup>;**

<sup>1</sup>Genetic Laboratory, Corporació Sanitària Parc Taulí, Sabadell, Spain, <sup>2</sup>Specialized Service in Health Mental and Intellectual Disability, Parc Hospitalari Martí i Julià, Salt (Girona), Spain, <sup>3</sup>Paediatric Service, Corporació Sanitària Parc Taulí, Sabadell, Spain.

Adults with unexplained intellectual disability (ID) have not been systematically addressed for genetic analysis. The goal of this study is to establish the genetic aetiology of 100 adult patients affected by dual diagnosis of mild (IQ=75-50) or moderate (IQ=50-35) ID and psychiatric/behavioural disorder. Different tests were used to evaluate cognitive and psychiatric profile. Patients were analyzed with a sequential genetic workflow, including molecular karyotype (array-CGH Agilent Technologies, 400K). Customized MLPA and FISH analyses were used to validate and determine the inheritance of pathogenic copy number variants (pCNV) and variants of unknown significance likely pathogenic (VOUSp).

We identified a genetic abnormality in 38 patients: 14 well known specific syndromes, 4 cases with chromosomal rearrangements and 20 cases with CNVs (8 pCNV and 12 VOUSp). A 2p16.2 deletion is the genetic basis in two

unrelated cases, describing a common cognitive, psychiatric and dysmorphic phenotype. Three unrelated patients present a 7q31 deletion as the putative genetic cause. A 15q14q15.1 duplication and an homozygous 3q29 duplication were identified in two independent siblings suggesting that both regions could be dosage sensitive.

Eleven genes included in PCNV or VOUSlp had been related to a wide range of psychiatric phenotypes: *NRXN1*, *IMMP2L*, *MSRA*, *SLC1A1*, *CTNNA3*, *SOX5*, *UBE3A*, *CHRNA7*, *SPRED1*, *PRKCA* and *SHANK3*. The CNVs contribute equally to both mild and moderate ID as well as to different psychiatric disorders even though the odds of having psychiatric co-morbidity was 4.22 times higher in patients with a causative CNV.

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#### P09.105

#### Diagnosing Neuronal ceroid lipofuscinosis with Next-generation sequencing, an example

*G. J. Braathen<sup>1</sup>, S. Slind<sup>2</sup>, Ø. L. Busk<sup>1</sup>, K. Tveten<sup>1</sup>, Ø. L. Holla<sup>1</sup>, H. T. Hilmarsen<sup>1</sup>, J. R. Helle<sup>1</sup>;*

<sup>1</sup>*Section of Medical Genetics, Department of Laboratory Medicine, Skien, Norway,*

<sup>2</sup>*Department of Pediatrics, Telemark Hospital, Skien, Norway.*

Introduction: The incidence of neuronal ceroid lipofuscinoses (NCL; CLN) in different countries vary between 0.5-3.9/100.000. NCLs are characterized by the intracellular accumulation of autofluorescent lipopigment storage material in different patterns ultrastructurally.

NCLs are clinically and genetically a heterogeneous group of neurodegenerative disorders. Originally, they were classified broadly according to age at onset. However with the identification of molecular defects, the NCLs are now classified numerically according to the underlying gene defect. NCL2 refers to NCL caused by mutation in the *CLN2* gene (*TPP1*), regardless of the age at onset.

Next-Generation Sequencing (NGS) is fairly quick and to a low cost compared with old methods when sequencing many genes simultaneously. Exome sequencing is a technique for sequencing all the protein-coding genes in a genome. It is also possible to filter out and investigate for only the genes of interest.

Methods: A five year old girl developed early sleep problems that abated and during second year of life speech problems having only few words. From three years of age she had seizures, frequent falls, sleep problems and emotional stress. Her 14 months old brother had sleep problems and cries.

Results: Filtered NGS exome for CLN genes identified a compound heterozygous mutation in the *TPP1* gene, i.e. NM\_000391.3 c.509-1G>A and c.622C>T p.(Arg208\*) consistent with the disorder NCL2.

Conclusion: These two variants have never been described together causing NCL2.

Filtered NGS exome is a powerful, cost- and time-effective tool to investigate all known genes causing NCLs.

Thus, MRI and eye investigation have been postponed.

#### P09.106

#### Genetic Contributions to Neuroticism-Related Brain Structures

*H. Kim<sup>1</sup>, H. Kang<sup>2</sup>, S. Kim<sup>1</sup>, Y. Chang<sup>3</sup>, S. Ryu<sup>3</sup>, H. Shin<sup>3</sup>, D. Lee<sup>2</sup>, H. Kim<sup>1</sup>;*

<sup>1</sup>*Ewha Womans University, Seoul, Korea, Republic of, <sup>2</sup>Seoul National University, Seoul, Korea, Republic of, <sup>3</sup>Sungkyunkwan University, Seoul, Korea, Republic of.*

Introduction: Neuroticism is a moderately heritable personality trait considered to be a risk factor for stress and impaired health as well as psychiatric disorders. Our aim in this study is to test whether neuroticism is associated with brain volumes and to identify single nucleotide polymorphisms (SNPs) associated to the neuroticism-related brain volumes.

Materials and Methods: In this study, we combined genetics, imaging and personality data obtained from 123 women between the ages of 21-64 from Kangbuk Samsung Cohort Study. A total of 5,862,302 SNPs were tested for association with brain volume in a genome-wide association study. We also investigated if the identified SNPs are operating as a eQTL and in which brain region using Braineac web-based resource.

Results: High neurotic individuals showed increased volumes in precuneus, superior parietal lobule, middle frontal gyrus, and superior frontal gyrus. None of the genetic markers reached genome-wide significance. However, SNPs near *ADSS* for superior parietal lobule, within *RBFOX1* for middle frontal gyrus, within *KCNH8* for superior frontal gyrus were showed suggestive p-values ( $< 1 \times 10^{-5}$ ). The genes were previously reported to be associated with neurological phenotypes. Furthermore, allelic differences in the SNPs were associated with differential mRNA expression in the cis-acting region. Conclusion: Our results could improve our conceptual framework of processes related to brain volume reduction and facilitate a better understanding

of neuroticism personality.

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#### P09.107

#### Exome sequencing identifies rare pathogenic NGLY1 mutations in a patient with developmental delay, seizures and hyperkinetic movement disorder

*H. Daoud<sup>1</sup>, J. Wagner<sup>2</sup>, O. Jarinova<sup>1</sup>, K. Boycott<sup>1,2</sup>, H. McMillan<sup>3</sup>, D. A. Dymant<sup>1,2</sup>;*

<sup>1</sup>*Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada,*

<sup>2</sup>*Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada, <sup>3</sup>Division of Neurology, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.*

Background: N-glycanase 1 is a conserved cytosolic enzyme that catalyzes the deglycosylation of misfolded N-linked glycoproteins, an essential step in the endoplasmic reticulum-associated degradation pathway (ERAD). N-glycanase 1 deficiency, caused by loss-of-function mutations in the *NGLY1* gene, is an extremely rare autosomal recessive disorder characterized by global developmental delay, movement disorder, hypotonia, liver dysfunction, and alacrima. To our knowledge, a total of 14 patients have been reported in the literature thus far.

Methods: We investigated a 23-month-old girl who presented with seizures, developmental delay, hypotonia, and hyperkinetic movement disorder. We performed whole Exome Sequencing (WES) in the patient and her parents using the Nextera Rapid Capture Exome kit (37 Mb) and the NextSeq 500 platform (Illumina).

Results: We identified compound heterozygous mutations in the *NGLY1* gene: a nonsense mutation c.A1201A>T (p.Arg401\*) inherited from the mother, and a splicing mutation c.1150-1G>C (p.?) inherited from the father. The nonsense mutation c.A1201A>T is the most common deleterious allele identified in patients with N-glycanase 1 deficiency. The splicing mutation c.1150-1G>C is novel and we show that it leads to an in-frame deletion (p.Asp335\_Val384del) that removes part of the transglutaminase-like (catalytic) domain of the protein.

Conclusion: We report here an additional patient with compound heterozygous mutations in the *NGLY1* gene detected by WES. The phenotype of our patient is consistent with the previously reported cases and further support that mutations in the *NGLY1* gene lead to a recognizable disorder.

#### P09.108

#### Next Generation Sequencing in Parkinson's disease - our experience

*I. Novakovic<sup>1</sup>, V. Dobricic<sup>2</sup>, M. Jankovic<sup>2</sup>, M. Svetel<sup>3</sup>, A. Maver<sup>4</sup>, B. Peterlin<sup>4</sup>, A. Westenberger<sup>5</sup>, C. Klein<sup>5</sup>, V. Kostic<sup>1</sup>;*

<sup>1</sup>*Faculty of Medicine, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Neurology Clinic CCS, Belgrade, Serbia, <sup>3</sup>Faculty of Medicine, University of Belgrade, Belgrade, Serbia, <sup>4</sup>Institute of Medical Genetics, CC Ljubljana, Ljubljana, Slovenia, <sup>5</sup>Institute of Neurogenetics, University of Luebeck, Luebeck, Germany.*

Parkinson's disease (PD) is the most common movement disorder, clinically characterized by resting tremor, bradykinesia and muscle rigidity. Key neuropathological substrate of PD is loss of dopaminergic neurons in basal ganglia and presence of intraneuronal Lewy bodies. Vaste majority of PD cases have multifactor etiology but in 10-15% patients monogenic pattern of inheritance is noticeable, with familiar occurrence and/or early onset of disease. In the past decades at least 18 PARK disease causing and susceptibility loci have been identified, but genetic basis of PD is not fully clarified. Recent Next Generation Sequencing (NGS) methods have their application in this field also. We describe here results of NGS implementation in five PD families from Serbia. These patients have been previously screened for the most common mutations in PARK loci (*LRRK2*, *PARKIN*, *PINK1*, *DJ1*, *GBA*, *VPS35*) by direct sequencing of target regions and MLPA method. NGS was performed on Illumina MiSeq platform using TruSight One panel. After bioinformatic analysis of NGS data we detected novel *TGM6* c.1076C>T (p.Pro359Leu) variant in early onset PD family with three affected members in two consecutive generations. Mutations in *TGM6* usually cause spinocerebellar ataxia type SCA35, but it has previously been shown that mutations in other SCA genes are clinically present with PD. In another PD family NGS revealed previously detected susceptibility variant N370S in *GBA* gene. In the remaining PD families NGS did not reveal significant pathogenic variants. Our results support complexity of PD genetics, and illustrate power and challenges of NGS methods in this field.

## P09.109

### Screening for Niemann-Pick type C disease among adult patients with neurodegenerative disorders of unknown etiology

C. Castro-Fernández<sup>1,2</sup>, P. Blanco-Arias<sup>1,3</sup>, C. García-Sancho<sup>4</sup>, R. Rodríguez<sup>5</sup>, V. Rodríguez-Sureda<sup>6</sup>, C. Pérez-Sousa<sup>4</sup>, M. J. Moreno-Carretero<sup>7</sup>, S. Mederer<sup>8</sup>, M. Alberte-Woodward<sup>9</sup>, M. Guijarro<sup>9</sup>, A. Sesar<sup>10</sup>, M. Arias<sup>1,10</sup>, M. J. Sobrido<sup>1,3</sup>,

<sup>1</sup>Instituto de Investigación Sanitaria, Santiago de Compostela, Spain, <sup>2</sup>Universidad de Santiago de Compostela, Santiago de Compostela, Spain, <sup>3</sup>Fundación Pública Galega de Medicina Xénómica, Santiago de Compostela, Spain, <sup>4</sup>Complejo Hospitalario Universitario, A Coruña, Spain, <sup>5</sup>Complejo Hospitalario Universitario, Ourense, Spain, <sup>6</sup>CIBBIM – Nanomedicina, Vall d'Hebron Institut de Recerca, Barcelona, Spain, <sup>7</sup>Complejo Hospitalario Universitario, Vigo, Spain, <sup>8</sup>Complejo Hospitalario, Pontevedra, Spain, <sup>9</sup>Hospital Universitario Lucus Augusti, Lugo, Spain, <sup>10</sup>Complejo Hospitalario Universitario, Santiago de Compostela, Spain.

**Introduction:** Niemann-Pick type C (NPC) is an autosomal recessive lysosomal disorder resulting from mutations in the NPC1 or NPC2 genes. Adult onset NPC has neuropsychiatric manifestations that can mimic several neurodegenerative diseases. **Aim:** Multicenter, transversal study to screen for NPC in adult patients with undiagnosed neurodegenerative disorders.

**Materials and Methods:** 1) Patient selection through retrospective revision of clinical records in neurology departments from Galicia (Spain). 2) Major inclusion criteria: progressive gait disorder, involuntary movements, supranuclear gaze palsy and cognitive decline. Secondary criteria: prolonged neonatal jaundice, visceral involvement, psychiatric symptoms, epilepsy, cataplexy and family history. 3) A Next Generation Sequencing panel was designed for Ion PGM® using AmpliSeq® Designer program with 99.8% coverage of NPC1 and NPC2. 4) Biochemical and cellular tests were performed in patients with suspected genetic variants.

**Results:** Eighteen patients (13 men, 5 women, ages 22-66) were included. Altogether, we identified five potentially pathogenic variants in NPC1 and one in NPC2. The diagnosis of NPC was confirmed in two cases with homozygous NPC1 mutations. One patient carrying only one splicing variant had a late disease onset and intermediate Filipin test result. Two patients had variants of as yet uncertain significance.

**Conclusions:** NPC should be considered in adults with neuropsychiatric disorders of unclear etiology. Analysis of gene panels via NGS provides an efficient diagnostic strategy. Genetic confirmation facilitates detection of additional family cases, allows early treatment and genetic counseling. The Filipin test aids in the interpretation of variants of uncertain significance.

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## P09.111

### The evolving NRXN1 deletion syndrome; reduced penetrance, variable expressivity and complex counselling

M. M. Al Shehhi<sup>1</sup>, J. E. Fitzgerald<sup>2</sup>, V. McInerney<sup>3</sup>, S. Shen<sup>4</sup>, D. R. Betts<sup>1</sup>, L. Mc Ardle<sup>1</sup>, K. Gorman<sup>4</sup>, M. King<sup>4</sup>, A. Green<sup>1</sup>, L. Gallagher<sup>2</sup>, S. A. Lynch<sup>4,5</sup>;

<sup>1</sup>Department of Clinical Genetics, OLCHC, Dublin, Ireland, <sup>2</sup>Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland, <sup>3</sup>Regenerative Medicine Institute, School of Medicine, NUI, Galway, Ireland, <sup>4</sup>Children's University Hospital, Temple St, Dublin, Ireland, <sup>5</sup>Academic Center on Rare Diseases, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland.

**Background:** Deletions or mutations of neurexin-1 (NRXN1) remain a difficult counselling issue because of reduced penetrance and variable expressivity. We have reviewed 33 families known to our center to estimate penetrance and document the phenotype.

**Method:** Cases were identified by database review within our center or through collaboration with colleagues. Genomic data, clinical phenotype & family history information was extracted and analyzed. Penetrance was calculated using the method proposed by Kirov et al, 2014. The unaffected carriers were used as controls.

**Results:** We identified 53 NRXN1 deletion carriers and one individual with a point mutation of whom 37(69%) had a definite phenotype. 33 were probands and the rest were first degree relatives. 87% of probands were investigated because of unexplained developmental delay mostly speech and language or learning difficulties and 63% had Autism. 17/53 were intronic deletions. The overall penetrance was ~66% for any phenotype and 40% for Autism. Congenital anomalies were reported in 7 infants and seizures in 6. **Conclusion:** Our data suggest that neurexin-1 deletions present with a variable phenotype and are not fully penetrant. Intronic deletions are pathogenic. Whilst, a parent that carries the deletion has a 50% risk of passing it on, the risk of a child with the deletion developing Autism is approximately 40%.

Genetic counselling remains problematic as the additional factors that trigger the phenotype are unclear and impossible to predict. Larger studies will help remove ascertainment bias. Further biological studies may help us understand the variability observed.

## P09.112

### Deconstructing obsessive-compulsive disorder by whole exome sequencing and rare variant association study

L. Domenech<sup>1</sup>, R. Rabionet<sup>1</sup>, G. Escaramís<sup>1</sup>, E. Real<sup>2</sup>, D. Trujillano<sup>1</sup>, H. Susak<sup>1</sup>, M. Bosio<sup>1</sup>, S. Ossowski<sup>1</sup>, A. Carracedo<sup>3</sup>, P. Alonso<sup>2</sup>, X. Estivill<sup>1</sup>;

<sup>1</sup>Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, <sup>2</sup>Hospital Universitari de Bellvitge, IDIBELL, L'Hospitalet de Llobregat, Spain, <sup>3</sup>Santiago de Compostela University, Santiago de Compostela, Spain.

Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that affects 1-3% of the population worldwide. It is listed as the tenth most disabling illnesses of any kind. Family and twin studies demonstrated that OCD involves both environmental and polygenic risk factors. However, despite an abundance of candidate genes, linkage studies and GWAS, there has been very little progress towards elucidating the genetic causes of this disorder. This project represents a novel strategy that will contribute to explain the aetiology of OCD.

We performed whole exome sequencing of 306 unrelated OCD cases followed by a rare variant association study. Specifically, we aggregated exonic non-synonymous and splicing variants into genes and tested for association with OCD using a group of control exomes from our in-house database. We accounted for minor allele frequencies (prioritizing rare variants) and pathogenicity score. After implementing this study, we identified a list of genes harbouring a higher number of mutations in cases compared to controls. We then implemented a partial least square analysis to identify combinations of mutated genes that can distinguish between OCD cases and controls. We also performed a pathway enrichment analysis with the significant genes, finding enriched pathways related with brain neurodevelopment or function. Finally, we selected some of these genes for further analysis, including functional studies of the identified variants.

Stronger evidence of association for the significant genes identified will be obtained through replication in larger cohorts of OCD.

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## P09.113

### Analysis of the role of copy number variation in Obsessive-compulsive disorder

D. Sanchez Chinchilla<sup>1</sup>, L. Domenech<sup>1</sup>, R. Rabionet<sup>1</sup>, G. Escaramís<sup>1</sup>, E. Real<sup>2</sup>, D. Trujillano<sup>1</sup>, H. Susak<sup>1</sup>, M. Bosio<sup>1</sup>, S. Ossowski<sup>1</sup>, P. Alonso<sup>2</sup>, X. Estivill<sup>1</sup>;

<sup>1</sup>Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, <sup>2</sup>Hospital Universitari Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Spain.

Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder ranked by the World Health Organization among the 10 most debilitating disorders. The lifetime prevalence of OCD has been estimated to approximately 2% and, despite molecular studies have identified several relevant genes, much additional research is needed to establish definitive causes of the condition. In this study we tried to identify copy number variations (CNVs) conferring risk of OCD.

We have performed exome-sequencing on 306 OCD cases and more than 1000 non-OCD samples (control as well as other non-psychiatric disorders). Both NimbleGen and Agilent commercial exome capture kits were used for sequencing. In order to identify CNVs, we analyzed the data with ClinCNV (an in-house pipeline for CNV detection from exome data). Currently, we have performed the CNV calls in the OCD samples and are analyzing the remaining samples.

In the OCD cases, we identified an average of 33 CNV per individual, ranging in size from 1 kb to 3,5 Mb. Some of the detected variants were overlapping with known common CNVs. Some rare variants included relevant genes involved in psychiatric disorders. We will perform this analysis in the non-OCD samples and follow-up with association analysis and validation of relevant regions by direct typing in these and additional samples.

This work was supported by MINECO grant agreement SAF2013-49108-R.

## P09.114

### Clinical and Genetic Features of PKAN Patients in a Tertiary Center in Turkey

N. H. Akcakaya<sup>1</sup>, S. U. Iseri<sup>1</sup>, E. Battaloglu<sup>2</sup>, P. Tekturk<sup>2</sup>, H. Hanagast<sup>4</sup>, M. Eraksoy<sup>2</sup>, U. Ozbek<sup>1</sup>, Z. Yapıcı<sup>3</sup>;

<sup>1</sup>Institute of Experimental Medicine (DETAE), Istanbul, Turkey, <sup>2</sup>Bogazici University, Department of Genetics, Istanbul, Turkey, <sup>3</sup>Istanbul University, Istanbul Medical Faculty, Department of Neurology, Division of Child Neurology, Istanbul, Turkey, <sup>4</sup>Istanbul University, Istanbul Medical Faculty, Department of Neurology, Division of Movement Disorders and Behavioral Neurology, Istanbul, Turkey.

**Introduction:** Pantothenate kinase-associated neurodegeneration (PKAN), also known as neurodegeneration with brain iron accumulation 1 (NBIA1),

is caused by mutations of PANK2 gene. The major clinical types of PKAN's are early onset-rapid progressive form and late onset-slow progressive form.

Purpose: We aim to discuss clinical and genetic findings of 19 PKAN patients.

Method: Nineteen patients with prominent extrapyramidal symptoms and "eye-of-the-tiger" sign on the MRI, with clinically diagnosed PKAN included to the study. All patients were screened for PANK2 mutations. Medical history, neurological examination were documented. To predict the potential effects of novel variations on the PANK2 protein structure or function software in silico tools; Mutation Taster, SIFT and Polyphen and GeneSplicer were used.

Results: Twelve patients had early onset-rapid progressive form and seven patients had late onset-slowly progressive form. The presenting features were dystonia and gait disturbance in early onset patients whereas late onset presenting symptoms were varied. Mutation screening reveals five novel and six previously reported mutations. Beside exonic missense mutations and deletions; intronic mutations were also identified. Except one patient, all cases harbored the mutations in homozygous state.

Conclusion: The current report is first patient series of PKAN from Turkey associated with five novel inherited PANK2 mutations and shows a clear-cut genotype-phenotype correlation with a highest rate of homozygosity.

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#### P09.115

#### Parkinson's disease: identification of new variations in protein and antisense genes

*M. Valente<sup>1</sup>, R. Zangaglia<sup>2</sup>, S. Gagliardi<sup>1</sup>, I. Palmieri<sup>1</sup>, I. Ricca<sup>1</sup>, S. Zucca<sup>1</sup>, C. Pacchetti<sup>2</sup>, C. Cereda<sup>1</sup>;*

<sup>1</sup>Center of Genomics and post-Genomics, C. Mondino National Institute of Neurology Foundation, Pavia, Italy, <sup>2</sup>Parkinson's Disease and Movement Disorders Unit, C. Mondino National Institute of Neurology Foundation, Pavia, Italy.

Background. Deep sequencing technologies have revolutionized genetic studies, prompting innovative theories for Parkinson's Disease (PD). In addition to canonical protein genes associated with PD, potentially relevant antisense (AS) genes have been discovered as PINK1-AS. Variations in AS genes may interfere with protein gene regulation, affecting PD phenotype expression.

Objective. Identification of new genomic variants in both protein and AS genes in PD patients and expression of sense and AS genes in peripheral blood mononuclear cells (PBMCs).

Methods. Next Generation Sequencing analysis has been performed in a cohort of 80 Italian PD patients. True Seq Custom Amplicon Illumina platform was composed by PARK2, PINK1, DJ-1, LRRK2, SNCA, UCHL1, EIF4G1, ATP13A2, VPS35 and GBA genes. AS and protein coding gene expression was verified via RT-PCR.

Results. Data analysis showed the presence of new non-synonymous mutations in PARK2, LRRK2, PINK1 and ATP13A2. For mutations in PARK2 (p.W447G, p.R191Q), LRRK2 (p.I1784F, p.L2425V) and PINK1 (p.A124V) the in silico prediction (PolyPhen2 and Mutation Tester) supported its pathological implication, while the mutation in ATP13A2 (p.N1091S) resulted neutral. Regarding new AS genes, we found a not yet described SNCA-AS, aside from PINK1-AS and UCHL1-AS. An increase expression of AS versus sense genes was reported for SNCA-AS and UCHL1-AS in PD patients; a similar trend was observed in controls.

Discussion. NGS data indicated variations both in protein and AS genes, opening intriguing perspectives in PD genetics. In PBMCs, the expression of AS genes significantly increase respect to sense genes. Moreover, variation in AS genes are specific for antisense.

#### P09.116

#### Alteration of the splicing forms of the 5'-UTR of DJ-1 (PARK7) in SNPs related to Parkinson disease in Basque population

*F. Cardona<sup>1,2</sup>, V. Andreu-Fernández<sup>1</sup>, J. Hernández-Baixauli<sup>1</sup>, A. Baquero-Vaquer<sup>1</sup>, I. Nebot<sup>1</sup>, J. Martí-Massó<sup>3,2</sup>, A. López de Munain<sup>3,2</sup>, J. Pérez-Tur<sup>1,2,4</sup>;*

<sup>1</sup>Unidad de Genética Molecular. Instituto de Biomedicina de Valencia. Consejo Superior de Investigaciones Científicas, Valencia, Spain, <sup>2</sup>CIBERNED, Madrid, Spain, <sup>3</sup>Instituto Biodonostia. Hospital Universitario Donostia, San Sebastián, Spain, <sup>4</sup>Unidad Mixta de Investigación en Neurología y Genética Molecular. Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain.

Introduction: DJ-1 (PARK7) codifies a chaperone with protease activity involved in stress response, particularly under oxidative conditions. This protein is also involved in mitochondria homeostasis, in collaboration with

Parkin and PINK1. Mutations in this gene have been associated to autosomal recessive Parkinson disease. In our group, genetic studies in a Basque population have shown a positive association of two single nucleotide polymorphisms (SNPs) near an area of alternative splicing in the 5'-UTR of DJ-1. Here we intend to determine how these SNPs affect the expression of the gene and if they affect the isoform ratio.

Material and methods: We used the exon trapping assay modified for exon 1, which does not contain naturally a splicing acceptor site. The quantification of the splicing forms was performed by RT-qPCR. To study the effect of the variants of the 5'-UTR in gene expression regulation, we used constructs with luciferase under the control of SV40 promoter and the different forms of the 5'-UTR of DJ-1. All the plasmids were transfected in SHSY-5Y cell line differentiated to dopaminergic neuronal phenotype, checking the differentiation by determination of the expression of tyrosine hydroxylase and by cell morphology.

Results: Preliminary studies show that the different alleles of these SNPs affect the ratio of the different splicing forms, and therefore the expression of the gene.

Conclusions: Two SNPs were found as associated to Parkinson disease in this population and the genotypes associated to the disease alter the correct expression of DJ-1.

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#### P09.118

#### Search for genetic markers involved in Impulse Control Disorder in Parkinson's Disease: preliminary findings.

*X. H. Castro Martínez<sup>1</sup>, C. Martínez-García<sup>2</sup>, P. J. García-Ruiz<sup>3</sup>, J. C. Martínez Castrillo<sup>4</sup>, L. Vela<sup>5</sup>, M. Mata<sup>6</sup>, I. Martínez-Torres<sup>7</sup>, F. Palau<sup>1</sup>, J. Hoenicka<sup>1</sup>;*

<sup>1</sup>Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Barcelona, Spain, <sup>2</sup>Centro de Investigación Príncipe Felipe, Valencia, Spain, <sup>3</sup>Fundación Jiménez Diaz, Madrid, Spain,

<sup>4</sup>IRYCIS, Hospital Universitario Ramón y Cajal, Madrid, Spain, <sup>5</sup>Hospital Universitario de Alcorcón, Alcorcón, Spain, <sup>6</sup>Hospital Universitario Infanta Sofía, Madrid, Spain, <sup>7</sup>Hospital Universitario La Fe, Valencia, Spain.

Introduction: Impulse control disorders (ICDs) comprise a wide spectrum of impulsive/compulsive behaviors frequently found in patients with Parkinson's disease (PD) receiving antiparkinsonian treatment. Here, we studied in PD patients three genes that code for proteins involved in meso-cortico-limbic circuit in the brain: i) ANKK1 for dopamine synthesis; ii) DRD3 for the dopaminergic receptor D3 and iii) SCL6A3 for the dopamine transporter.

Methods: Up to date, 33 early-onset PD patients (age below 45) have been recruited from the Spanish ICD Multicenter Study. ICD were assessed using the QUIP questionnaire. This test has been validated for each ICD with a potentially addictive reinforcement (ICDARs) as well as for funding.

Results: In the early-onset PD sample several tendencies were appreciated (Table): i) ANKK1, there is an overrepresentation of the A1+ genotype not only in the total sample ( $p=0.056$ ) but also in ICD+, ICDAR and funding; ii) DRD3, the homozygous Ser9/Ser9 genotype seems to be protective factor, and iii) SCL6A3, the A9+ genotype seems to be a risk factor for ICD+ and funding. By contrast, in the classical PD sample, none of the genes were associated with the exception of SCL6A3 that showed the A9+ genotype overrepresented in the total sample ( $p=0.05$ ).

Conclusion: Genetic factors associated with ICD in PD may be different depending on the age of onset.

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Gene/poly-morphism	Geno-type	Cont-rols % (N)	Genetics of early-onset PD				
			PD % (N)	PD/ICD- % (N)	PD/ICD+ % (N)	PD/ICDAR+ % (N)	PD/Punding+ % (N)
ANKK1/ TaqIA	A1+	28.6(82)	45.5(15)	33.3(5)	52.9(9)	54.5(6)	50.0(5)
	A1-	71.4(205)	54.5(18)	66.7(10)	47.1(8)	45.5(5)	50.0(5)
DRD3/ Ser9Gly	TT	48(138)	60.6(20)	73(11)	50(9)	50(6)	50(5)
	CT	40(110)	33.33(11)	27(4)	39(7)	42(5)	40(4)
SCL6A3/ VNTR	CC	12(39)	6.0(2)	0(0)	11(2)	8(1)	10(1)
	A9-	50.4(138)	45.43(15)	53(8)	39(7)	42(5)	30(3)
SCL6A3/ VNTR	A9+	49.6(136)	54.54(18)	47(7)	61(11)	58(7)	70(7)

## P09.119

**LRRK2, GBA and SMPD1 founder mutations and Parkinson's disease in Ashkenazi Jews**

*E. Dagan<sup>1</sup>, I. Schlesinger<sup>2,3</sup>, A. Kurolop<sup>4,3</sup>, M. Ayoub<sup>4</sup>, M. Nassar<sup>2</sup>, J. Peretz-Aharon<sup>2,3</sup>, R. Gershoni-Baruch<sup>4,3</sup>;*

<sup>1</sup>Department of Nursing, University of Haifa, Haifa, Israel, <sup>2</sup>Department of Neurology and the Cognitive Neurology Institute, Rambam Health Care Campus, Haifa, Israel, <sup>3</sup>The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel, <sup>4</sup>Institute of Human Genetics, Rambam Health Care Campus, Haifa, Israel.

**Background:** Parkinson's disease (PD) has been associated with mutations in leucine-rich repeat kinase 2 (LRRK2), glucosidase beta acid (GBA), and sphingomyelin phosphodiesterase 1 (SMPD1), more so in the Ashkenazi Jewish population. Here we describe the clinical characteristics of 271 PD patients and compare carriers of Ashkenazi founder mutations in the aforementioned genes to PD patients who are non-carriers of such mutations.

**Methods:** The study cohort is composed of 271 Ashkenazi PD patients, previously reported by us (Dagan et al., 2015). Here we compare the clinical characteristics of PD patients stratified according to their carriage of founder mutations in the three causative genes (namely, SMPD1, GBA and LRRK2). PD patients, homozygotes for mutations in either GBA or LRRK2 and those who carried mutations in two causative genes were excluded.

**Results:** Six (2.2%), 54 (19.9%), and 22 (8.1%) PD patients carried mutation in SMPD1, GBA or LRRK2, respectively. Post hoc analysis singled GBA carriers as having significantly earlier age at onset and two-sided clinical manifestation at diagnosis compared to all other PD groups - SMPD1 and LRRK2 carriers and non-carriers. Other clinical manifestations were comparable between studied groups.

**Conclusion:** The overall clinical characteristics of PD patients carrying SMPD1, GBA, and LRRK2 mutations were similar to those of non-carriers. Although only GBA carriers demonstrate statistically significant earlier age of onset compared to non-carriers, it appears that LRRK2 and SMPD1 mutation carriers may reach significance with larger group numbers.

## P09.120

**Association of functional RAGE gene polymorphisms in the pathogenesis of inflammatory with Parkinson's disease in Turkish population**

*H. Aslan<sup>1</sup>, O. Cilingir<sup>1</sup>, M. Ozdemir<sup>1</sup>, B. Durak Aras<sup>1</sup>, O. Kutlay<sup>1</sup>, E. Erzurumluoglu<sup>1</sup>, A. Afagh<sup>1</sup>, M. Akinci<sup>2</sup>, S. Ozkan<sup>2</sup>, S. Artan<sup>1</sup>;*

<sup>1</sup>Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Genetics, Eskisehir, Turkey, <sup>2</sup>Eskisehir Osmangazi University, Faculty of Medicine, Department of Neurology, Eskisehir, Turkey.

Parkinson Disease (PD) is a severe, progressive neurodegenerative disease. It is reported that genes responsible for the etiology of PD leads to the disease by the pathological mechanisms which cause neuron damages. A large number of polymorphic genetic changes are also known to pose a risk for Parkinson's disease.

Receptor for advanced glycation end-products (RAGE) gene encodes a protein which is expressed in microglial cells and this protein is also expressed in the central nervous system. Two distinct polymorphisms (374T>A, -429T>C and 63 bp Ins/Del polymorphisms) affect the RAGE expression. G82S polymorphism occurs as result of transition of serine in preference to glycine at the 82nd of the RAGE gene. The resulting polymorphic changes show an increase in ligand binding and downstream signaling.

The present study aims to investigate the relationship of -429T>C, -374T>A, 82G>S and 63 bp Ins/Del polymorphisms with PD. A total of 174 PD patients and 150 healthy-matched individuals in Turkish population were registered. Herewith, PCR-RFLP and ARMS methods were employed in order to be able to determine polymorphisms.

429T>C, -374T>A, and 82G>S polymorphisms showed significant differences between PD patients and controls ( $p < 0.001$ ,  $p < 0.05$ ,  $P < 0.001$ , respectively). However, no significant difference in the 63 bp Ins/Del polymorphisms could be seen between the groups. In conclusion 429T>C, -374T>A, and 82G>S polymorphisms may be related with inflammatory pathogenesis of PD. Since the analysis of these polymorphisms in PD is the first study in Caucasian population, the distribution of the alleles may shed light on future studies.

## P09.121

**Partial tandem duplication of the PDCD10 gene is a new mechanism of familial cerebral cavernous malformation**

*E. Borràs<sup>1</sup>, I. Hernan<sup>1</sup>, M. Gamundi<sup>1</sup>, B. Mañé<sup>1</sup>, B. Pascual<sup>1</sup>, M. Font<sup>2</sup>, D. Armengol<sup>3</sup>, M. Carballo<sup>1</sup>;*

<sup>1</sup>Unitat de Genètica Molecular, Hospital de Terrassa, Terrassa, Spain, <sup>2</sup>Servei de Neurologia, Hospital de Terrassa, Terrassa, Spain, <sup>3</sup>Servei de Medicina Interna, Hospital de Terrassa, Terrassa, Spain.

Introduction: Familial cerebral cavernous malformation (CCM) is inheri-

ted as an autosomal dominant trait and it is caused by mutations in any of three genes involved in vascular morphogenesis and/or remodelling: KRIT1 (40%), CCM2 (20%) and PDCD10 (10-20%). Up to date PDCD10 mutations include partial or whole gene deletion, nonsense and splicing mutations. Here we report the first case of a partial tandem duplication of PDCD10 causing familial CCM.

**Materials and methods:** A 38-year-old male patient presented with paresthesias/dysesthesias in the left hemibody and referred pain with crural predominance. Cranial MRI revealed multiple CCM with probable complicated right frontal cavernoma. After discarding a common small deletion in CCM2, we performed MLPA analysis of the three CCM genes. Findings were confirmed by long-range PCR using primers designed to detect a tandem duplication of PDCD10 exons 6-7, followed by primer-walking sequencing and analysis of mRNA expression.

**Results:** The patient carried a tandem duplication of PDCD10 exons 6-7, probably in a mosaic state according to MLPA ratios. Primer-walking defined rearrangement junctions in introns 5 and 7, and identified the ends of the 7812-bp duplication. Consistently, an aberrant PDCD10 minor transcript with a 245-bp insertion, corresponding to duplicated exons 6-7, was identified by cDNA amplification.

**Discussion:** Duplication of PDCD10 exons 6-7 causes a frameshift and a premature stop codon (p.Asp133Leufs\*14) that results in a loss-of-function allele. Therefore, this novel mechanism should be taken into account when performing mutation screening in CCM.

## P09.122

**Novel PIGN mutations in a patient with multiple congenital anomalies hypotonia seizures syndrome**

*K. Stouffs<sup>1,2</sup>, S. Seneca<sup>1,2</sup>, A. Jansen<sup>3,2</sup>, A. Gheldof<sup>1,2</sup>, M. Bonduelle<sup>1</sup>, K. Keymolen<sup>1</sup>, L. De Meirlier<sup>3,2</sup>;*

<sup>1</sup>Center for Medical Genetics, UZ Brussel, Brussels, Belgium, <sup>2</sup>Neurogenetics Research Group, Reproduction Genetics and Regenerative Medicine Research Group, Vrije Universiteit Brussel, Brussels, Belgium, <sup>3</sup>Paediatric Neurology Unit, Department of Paediatrics, UZ Brussel, Brussels, Belgium.

**Introduction:** Mutations in the phosphatidylinositol-glycan biosynthesis class N (PIGN) gene are causing defects in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway. Problems in this pathway are associated with multiple autosomal recessive disorders, often involving the central nervous system. Mutations in PIGN are causing 'multiple congenital anomalies hypotonia seizures syndrome 1' (MACHS1).

**Materials and Methods:** Exome sequencing was performed in an 12-year old child from non-consanguineous parents from Belgian/Peruvian origin. The girl presented with early onset hypotonia and developmental delay. She had dysmorphic features including a flat nasal bridge and prominent nose. Over the years she remained severely delayed and hypotonic with choreo-athetotic movements and generalized epilepsy, responsive to treatment. ERG was abnormal. The MRI of the brain showed progressive cortical atrophy.

**Results:** Exome sequencing showed the presence of biallelic variants, c.1158delG, p.Leu386Phefs\*17 and c.T956G, p.Val319Gly in the PIGN gene (RefSeq: NM\_012327.5) as Sanger sequencing analysis of the parents showed the independent segregation of the two observed alterations.

**Conclusions:** Exome sequencing showed the presence of two novel changes in the PIGN gene causing MACHS1. The c.T956G alteration is predicted to be pathogenic (SIFT score 0 and PolyPhen score 0.986); while c.1158delG change is completely disrupting the protein. Consequently, MACHS1 in this patient has been confirmed.

## P09.123

**Primary microcephaly (PM) and primordial microcephalic dwarfism: screening for gene mutations in a large series of 450 patients**

*S. Drunat<sup>1,2</sup>, S. Passemard<sup>3,2</sup>, Y. Vial<sup>1</sup>, N. Pouvreau<sup>1</sup>, H. Cavé<sup>1,4</sup>, A. Verloes<sup>3,2</sup>;*

<sup>1</sup>Molecular Genetics-Robert Debré Hospital, Paris, France, <sup>2</sup>U1141, Paris, France, <sup>3</sup>Clinical Genetics-Robert Debré Hospital, Paris, France, <sup>4</sup>U1131, Paris, France.

PMs are rare autosomal recessive disorders with a cumulate incidence from 1/10.000 to 1/100.000 births, depending on geographic origin. Five groups of PM are usually distinguished: Microcephaly, Primitive Hereditary, primary microcephaly with chorioretinopathy, and primary microcephalic dwarfisms: Seckel syndrome, microcephalic osteodysplastic dwarfism type 2, and Meier-Gorlin syndrome. PMs are characterized by an occipito-frontal head circumference more than 2 SD below the mean for sex, age, and ethnicity at birth, and at least below - 3 SD after six months. PMs result from mutations in more than 30 genes, considerably complicating molecular diagnostics. Therefore, due to the great clinical and genetic heterogeneity, two third to one half of PM patients have no identified gene mutation.

Mutation screening was performed in 450 patients with PM by targeted Sanger sequencing, panel gene NGS or exome sequencing. Causative mutations were found in 86 patients (19%). The majority of mutations were found in ASPM (34/86), followed by WDR62 (10/86) and MCPH1 (10/86). We also identified mutations in a further 19 genes (CDK5RAP2, CEP152, CEP135, PCNT, STIL, CENPJ, TUBGCP6, EFTUD2, TUBGCP4, RNU4ATAC, CIT, WDR81, DYRK1A, LIG4, RECQL3, MECP2, TUBA1A, KIF11 and PHGDH). Furthermore, by exome sequencing, we highlight some unexpected diagnoses, missed because the patient's phenotype lacks the typical clinical picture.

With mutations found in more than 20 genes, this study illustrates the extreme genetic heterogeneity of PMs. Due to phenotypic heterogeneity and atypical clinical presentation in some cases, exome sequencing for targeted gene panel negative patients is an efficient strategy to improve the diagnosis yield.

#### P09.124

#### D178N-129V Val genotype in a family with Creutzfeldt-Jakob Disease

Ö. Türedi<sup>1</sup>, O. Çilingir<sup>2</sup>, M. Aksu<sup>3</sup>, E. Gümüş<sup>2</sup>, H. Aslan<sup>2</sup>, S. Artan<sup>2</sup>, H. Karabulut<sup>4</sup>;

<sup>1</sup>Ankara University, Ankara, Turkey, <sup>2</sup>Osmangazi University, Eskisehir, Turkey, <sup>3</sup>Acibadem Kayseri Hospital, Acibadem University, Istanbul, Turkey.

**Introduction:** Prion diseases are a heterogeneous group of fatal neurological disorders which leads to rapid progressive neurodegeneration. 10-15% of cases are caused by pathological mutations of the normal prion protein gene (PRNP) and D178N- 129V haplotype determines Creutzfeldt-Jakob phenotype.

**Materials and Methods:** We present here a 53-year-old woman whose disease started with memory deficits, difficulty in instrumental activities, progressive cognitive impairment and ataxia. The clinical picture slowly worsened to a state of akinetic mutism in about 5 years. FLAIR and T2-weighted magnetic resonance imaging documented signal hyperintensity in the head of the caudate nucleus, inferior temporal lobe and insula. Testing for PRNP mutations was performed on genomic DNA sample. Since Huntington disease has similar symptoms, HTT gene CAG repeat size was also investigated. **Results:** The patient was found to be negative for HTT mutation. PRNP analysis revealed a GAC to AAC mutation at codon 178 resulting in aspartic acid to asparagine substitution, and homozygosity for valine at codon 129. The patient's father and mother were third degree cousins and her father, uncle and two aunts died a history of a neurological disorder very similar to that of the proband, and her paternal and maternal grandmother died after a rapidly progressive dementia. Molecular genetic data are not available for them. But the patient's brother and three cousins had the same mutation with the patient.

**Conclusion:** Clinical diagnosis of Creutzfeldt-Jakob disease is still challenging for most clinicians and therefore genetic testing should be performed where available for both diagnosis and genetic counselling.

#### P09.125

#### Mutations in RAB12 alter the TfR signaling pathway

E. Koschmidder<sup>1</sup>, A. Rakovic<sup>1</sup>, F. Borngräber<sup>1</sup>, A. Schmidt<sup>1,2</sup>, I. Bräne<sup>3</sup>, S. Schaake<sup>1</sup>, D. Alvarez-Fischer<sup>1</sup>, A. Weissbach<sup>1</sup>, H. Jabusch<sup>4,5</sup>, E. Altenmüller<sup>5</sup>, C. Klein<sup>1</sup>, K. Lohmann<sup>1</sup>;

<sup>1</sup>Institute of Neurogenetics, Lübeck, Germany, <sup>2</sup>Kurt Singer Institute for Music Physiology and Musicians' Health, Berlin, Germany, <sup>3</sup>Institute for Integrative and Experimental Genomics, Lübeck, Germany, <sup>4</sup>Institute of Musicians' Medicine, Dresden, Germany, <sup>5</sup>Institute of Music Physiology and Musicians' Medicine, Hannover, Germany.

**Introduction:** We recently identified two missense mutations (p.I196V; p.G13D) in RAB12 in patients with musician's dystonia (MD) by exome and genome sequencing.

RAB12 encodes a small GTPase that has been linked to lysosomal degradation of the transferrin receptor (TfR). TfR regulates the mitochondrial network integrity via a signaling pathway involving Mitofusin 2 (Mfn2). We investigated the effect of RAB12 mutations on this pathway.

**Materials and methods:** For functional characterization of RAB12 mutants, we used three different cellular models: 1) fibroblasts of two MD patients carrying I196V, 2) fibroblasts of healthy subjects overexpressing RAB12 wildtype (WT), G13D, and I196V, and 3) SH-SY5Y cells also overexpressing RAB12 WT, G13D, and I196V. GTPase activity, subcellular RAB12 localization, TfR degradation, Mfn2 protein levels, and the mitochondrial network were investigated.

**Results:** Elevated GTPase activity was detected in both mutants. Immunofluorescent staining revealed colocalization of ectopically expressed RAB12 with lysosomes and an altered subcellular distribution of both RAB12 mutants. There was no significant effect on TfR degradation or Mfn2 levels under basal conditions. However, we detected a reduction of Mfn2 in the G13D mutant compared to WT upon induction of oxidative stress. Fi-

nally, increased branching of the mitochondrial network was observed in fibroblasts overexpressing mutated RAB12 (I196V>G13D) compared to WT. **Conclusion:** Mutations in RAB12 lead to an altered protein function as demonstrated by changes in GTPase activity, subcellular localization of RAB12, and mitochondrial network. The mitochondrial integrity may be disturbed in mutant cells independently of TfR degradation.

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#### P09.126

#### The role of MEIS1 and SKOR1 in restless legs syndrome

F. Sarayloo<sup>1,2</sup>, A. Grant<sup>2</sup>, H. Catoire<sup>2</sup>, D. Rocheleau<sup>2</sup>, A. Dangoumau<sup>2</sup>, P. A. Dion<sup>2,3</sup>, G. A. Rocheleau<sup>2,3</sup>;

<sup>1</sup>Department of Human Genetics, McGill University, Montreal, QC, Canada, <sup>2</sup>Montreal Neurological Institute, McGill University, Montreal, QC, Canada, <sup>3</sup>Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada.

Restless legs syndrome (RLS), a sleep-related sensory-motor disorder, is characterized by an irresistible desire to move the legs because of abnormal sensations, induced in the evening by rest or inactivity and are partially relieved by movement. This common disorder has a prevalence of up to 15% in western populations. A successful GWAS on RLS patients identified common variants in intron 8 MEIS1 and intergenic region between MAP2K5/SKOR1 genes associated with RLS. These results were also replicated by other independent studies. Our group subsequently showed that MEIS1 risk haplotype is associated with decreased mRNA and protein expression of this gene. We examined the SKOR1 mRNA expression in patient cells with the MEIS1 risk haplotype using q-RT-PCR. A significant decrease in SKOR1 mRNA expression level was observed. A luciferase reporter assay using SKOR1 promoter region in HeLa cells with down regulated MEIS1 expression (using siRNA), followed by an EMSA using three fragments of SKOR1 promoter region was performed. The results suggest a direct interaction between MEIS1 and SKOR1. Given the transcriptional regulatory function of these genes, we are overexpressing or knocking out MEIS1 and SKOR1 genes, separately in human cell lines. Whole transcriptome of these sets of cells as well as control cells with normal endogenous expression of the genes will be extracted to perform RNA-Seq experiment. We believe that differential expression analysis of RNA-Seq data will lead to identify the genes that are in fact regulated by these transcription factors, some of which might have direct roles in RLS pathological pathways.

#### P09.127

#### Genetic diagnosis of patients with overlapping clinic Rett-like by targeted panel of genes

N. Brandi Tarrau<sup>1</sup>, P. Pacheco Fernández<sup>1</sup>, S. Vidal Falco<sup>2</sup>, L. Blasco Pérez<sup>2</sup>, A. García Cazorla<sup>1</sup>, M. o'Callaghan<sup>1</sup>, C. Ortez González<sup>1</sup>, M. Poo Arguelles<sup>1</sup>, E. Gean Molins<sup>1</sup>, M. Pineda Marfa<sup>2</sup>, J. García Peñas<sup>3</sup>, M. Guitart<sup>4</sup>, J. Armstrong Morón<sup>1</sup>;

<sup>1</sup>Hospital Universitario San Juan de Dios, Barcelona, Spain, <sup>2</sup>Fundación San Juan de Dios, Barcelona, Spain, <sup>3</sup>Hospital Infantil Universitario Niño Jesús, Madrid, Spain,

<sup>4</sup>Corporación Sanitaria Parc Taulí, Barcelona, Spain.

**Introduction:** It has been studied patients with clinical Rett (RTT) without genetic diagnosis using the Next Generation Sequencing (NGS). This type of diseases requires clinical diagnosis. The finding of a mutation confirms the diagnosis, but not necessarily established it.

NGS using targeted panel of genes facilitates the simultaneous study of causative genes of RTT and others whose mutation produces a similar or overlapping clinic, such Pitt Hopkins and Ohtahara syndromes.

**Material and Methods:** It has been designed a gene panel of 17 genes related to the clinical RTT-like presentation by HaloPlex Target technology. Enrichment System, for Illumina Sequencing.

Sanger sequencing was used in exons not well covered. If do not find any change, MLPA was done by causative RTT genes.

**Results:** We have detected mutations in genes that do not cause RTT pathology in 14 of 187 studied patients with clinical Rett-like. A total of 8 patients presented mutations in STBX1 gene, related with Ohtahara syndrome and 6 patients were redirected as a Pitt-Hopkins, finding mutations in causative TCF4 gene.

The database HGMD-professional, dbSNP, 1000 G and predictions pathology programs (Polyphen 2.0 and SIFT) were consulted.

NGS variants have been verified by Sanger sequencing and studied the origin of the mutation in the parents.

**Conclusion:** The genetic study by NGS allows to study a larger number of genes associated with RTT simultaneously, redirecting genetic diagnosis to other syndromes. Significantly reduce response time and the cost of the study.

## P09.128

### Discovery of new genes in Rett syndrome patients by WES

*S. Vidal Falcó<sup>1</sup>, M. Lucariello<sup>2</sup>, E. Vidal Ocabó<sup>3</sup>, N. Brandi Tarrau<sup>3</sup>, E. Gerotina Mora<sup>4</sup>, P. Jurado<sup>2</sup>, M. Esteller Badosa<sup>4,2</sup>, M. Pineda Marfa<sup>1</sup>, J. Armstrong Morón<sup>2</sup>,  
<sup>1</sup>Fundació Sant Joan de Déu, Barcelona, Spain, <sup>2</sup>Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain, <sup>3</sup>Hospital Universitari Sant Joan de Déu, Barcelona, Spain, <sup>4</sup>Cancer Epigenetics and Biology Program (PEBC), Barcelona, Spain.*

**Introduction:** Rett syndrome (RTT) is a developmental disorder of early onset, genetic basis, dominant inheritance and X-linked. There are described three genes that cause RTT: MECP2, CDKL5 and FOXG1. However, the etiology of 15% of RTT patients still remains unknown. Thus, the aim of this project is to identify new candidate genes in a cohort of patients with RTT phenotype without genetic diagnosis by Whole Exome Sequencing (WES).  
**Material and Method:** The patient and healthy parents without genetic diagnosis and negative CGHarray Cytoarray Plus (180K) (Agilent Microarrays) were analyzed by WES with TruSeq Sample Preparation Kit (Illumina). The filtering criteria used were: search mutations with 1000g MAF below 0.05 in genes with dominant inheritance, de novo, X-linked, autosomal subject to imprinting and/or with functional impact in the CNS. For the validated mutations in genes related with gabaergic pathways (SLC6A1 and GABBR2), we performed RT-qPCR (TaqMan Gene Expression) and Western Blotting assay of RNA and protein extraction from peripheral blood.

**Results:** Most of the validated mutations are genes expressed in the central nervous system: ion channels and GABA/glutamate/acetylcholine pathways. The preliminary studies of the SLC6A1 and GABBR2 expression were not conclusive.

**Conclusions:** We do not only identify 1 gene which causes RTT-like phenotype. Pathway of genes has to be address to understand overlapping phenotype, instead to one disease only. Although blood tissue has convenient extraction, we cannot detect RNA and protein of these genes. Our next studies are performing RT-qPCR and Western Blotting assays with RNA and protein extraction from fibroblasts.

## P09.129

### Study the effect of *Melilotus officinalis* extract on expression of *Daxx*, *Nfkb*, *Vegf*, *Syp*, *Psen1*, *Mapk3*, *Mtap2* and *Tnf* genes in the streptozotocin-rat model of sporadic Alzheimer's disease

*N. - Bazazzadegan<sup>1</sup>, P. Daneshmand<sup>1</sup>, M. Dehghan Shasaltaneh<sup>2</sup>, K. Saliminejad<sup>3</sup>, K. Kamali<sup>3</sup>, M. Banan<sup>1</sup>, H. Khorram Khorshid<sup>1</sup>,  
<sup>1</sup>Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Laboratory of Neuro-organic Chemistry, Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran, Islamic Republic of, <sup>3</sup>Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran, Islamic Republic of.*

Sporadic Alzheimer's disease (SAD) is a multi-factorial disease caused by genetic, epigenetic, environmental and metabolic factors. Current understandings of the possible mechanisms of AD such as inflammation and oxidative stresses in the brain have led us to investigation of potential AD therapeutics. Currently herbal medicines with few side effects are in the point of attention. *Melilotus officinalis* is a herbal extract with possible role as an anti-inflammatory and anti-oxidant agent that can improve the blood circulation. Among genes that have been implicated in SAD, eight genes including *Daxx*, *Nfkb*, *Vegf*, *Syp*, *Psen1*, *Mapk3*, *Mtap2* and *Tnf-α* have shown significant statistical diversity in Alzheimer human brain and STZ rat model. With this knowledge these genes have been chosen to be investigated for neuroprotective effect of *Melilotus officinalis* extract. This study was performed by comparing the expression level of genes in the hippocampus of SAD rat model using qPCR in treated and untreated groups. The therapeutic effect was studied at the behavioral, learning and memory level using Morris Water Maze (MWM) test as well. After determination of gene expression in the mentioned groups using qPCR technique, the higher expression in *Syp*, *Tnf*, *Mapk3* and lower expression in *Daxx*, *Nfkb*, *Vegf*, *Psen1*, *Mtap2* were identified in SAD rat's model treated with herbal extract. To determine the significance of changes, using statistical analyses is underway. In MWM, the significant changes in spatial learning that had been observed in rat's model group did not show any alteration in the herbal-treated group.

## P09.130

### Identification of a possibly polyadenylation mutation in STUB1 in SCAR16 without hypogonadism and spasticity by combining homozygosity mapping and exome sequencing

*B. Turkgenç<sup>1,2</sup>, M. Ozkilinc<sup>3</sup>, B. Sanlidag<sup>4</sup>, A. Eker<sup>5</sup>, M. C. Yakicier<sup>6</sup>, A. Tolun<sup>7</sup>, & G. Temel<sup>8,9</sup>,  
<sup>1</sup>Acıbadem Genetic Diagnostic Center, Istanbul, Turkey, <sup>2</sup>Marmara University, Department of Medical Biology and Genetics, Istanbul, Turkey, <sup>3</sup>İstanbul Technical University, Department of Molecular Biology and Genetics, Istanbul, Turkey, <sup>4</sup>Near East University, Department of Pediatrics, Nicosia, Cyprus, <sup>5</sup>Near East University, Department of Neurology, Nicosia, Cyprus, <sup>6</sup>Acıbadem University, Department of Molecular Biology and Genetics, Istanbul, Turkey, <sup>7</sup>Boğaziçi University, Department of Molecular Biology*

*and Genetics, Istanbul, Turkey, <sup>8</sup>Near East University, Department of Histology and Embryology, Nicosia, Cyprus, <sup>9</sup>Uludağ University, Department of Histology and Embryology, Bursa, Turkey.*

Autosomal recessive spinocerebellar ataxia-16 (SCAR16) is a rare progressive neurologic disorder characterized by truncal and limb ataxia resulting in gait instability. We studied a consanguineous family with three affected siblings presenting with cerebellar ataxia as the main and initial features plus variable other features. The age of onset was 57 years for the male and 31 and 37 for the females. All patients showed marked cerebellar atrophy on MRI. Further, clinical signs of pyramidal tract damage with increased lower limb tendon reflexes were observed, but no Babinski response, ankle clonus, lower limb spasticity or hypogonadism was evident. The females were unable to walk whereas the male had only moderate walking disability. One of the females had a relatively more progressive disease course and additionally cognitive impairment and nystagmus. Homozygosity mapping on three patients followed by exome sequencing on one patient revealed a novel homozygous c.\*240T>C variant in the 3' UTR of STUB1 gene. The variant is absent in all public databases, is highly conserved among species and most likely disrupts the polyadenylation signal in the mRNA, as indicated by online tool DNA Poly(A) Signal Miner. In contrast to the previous reports, our findings show that STUB1-ataxia can start even after age 50, and neither hypogonadism nor spasticity is an obligatory feature. Also, the family is a rare example of a neurologic disease with more severe manifestation in females. Functional studies are ongoing to investigate the mechanism of pathogenicity resulting from the mutation.

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## P09.131

### Neurodevelopmental copy number variants are enriched for novel schizophrenia risk factors

*E. Rees, K. Kendall, V. Escott-Price, A. Pocklington, P. Holmans, M. O'Donovan, M. Owen, G. Kirov, J. Walters;  
Cardiff University, Cardiff, United Kingdom.*

**Introduction:** At least 11 rare copy number variants (CNVs) have been shown to be major risk factors for schizophrenia. These CNVs also increase risk for other early onset neurodevelopmental disorders (NDs). We hypothesised that additional CNVs associated with NDs are likely to be hitherto undiscovered schizophrenia risk factors.

**Methods:** We analysed 50 ND CNVs that have not been associated with schizophrenia in a new sample of 6,934 schizophrenia cases and 8,751 controls, combined with previously published datasets, for a total of 20,403 schizophrenia cases and 26,628 controls.

**Results:** Collectively, these ND CNVs were significantly enriched for schizophrenia. ( $P=1.01 \times 10^{-6}$ ,  $OR=1.90$ ). 19 CNVs have increased ORs for schizophrenia, compared to only 4 with ORs  $< 1$ , and no observations were made among cases or controls at the remaining 27 loci. For individual loci, 16p12.1 deletions are significantly associated with schizophrenia risk after correction for multiple testing (case rate=0.16%, control rate=0.045%,  $q$ -value=0.017,  $OR=3.3$ ). Two additional new loci reached nominal levels of statistical significance (deletions at 2q11.2 and duplications at 10q11.21q11.23). Our results suggest a large proportion of the 50 loci are likely to be novel risk factors for schizophrenia, but due to their rarity, the available sample size precludes statistical confirmation. Additionally, analysis of our new dataset alone provides independent support for the 11 known schizophrenia risk loci.

**Conclusions:** We provide evidence for the existence of additional schizophrenia CNV loci, identify deletions of 16p12.1 as a novel schizophrenia risk factor, and strengthen the support for an aetiological overlap between neurodevelopmental disorders.

## P09.132

### The neurodevelopmental mechanisms of schizophrenia: input of maternal genes

*I. Halayenka<sup>1</sup>, N. Danilenko<sup>1</sup>, E. Aksyonova<sup>1</sup>, A. Liaudanski<sup>1</sup>, V. Objedkov<sup>2</sup>, A. Kandratsenka<sup>1</sup>, N. Drobot<sup>1</sup>, M. G. Siniavskaya<sup>1</sup>, O. Levadanskaya<sup>1</sup>, O. Davydenko<sup>1</sup>,  
<sup>1</sup>Institute of Genetics and Cytology, Minsk, Belarus, <sup>2</sup>Belarusian Medical State University, Minsk, Belarus.*

**Introduction:** According to the hypothesis of neurodevelopment of schizophrenia, the causes of the pathology are hidden in early stages of brain development, long before the disease manifestation. The idea that mother bears genetic predisposition to the development of different fetal anomalies due to various complications of pregnancy can explain the link between these unfavorable maternal situations and development of schizophrenia in progeny.

**Materials and Methods:** The aim of our research was to compare polymorphic allele rates of inflammation: TNF $\alpha$  (-308 G/A), IL-6 (-174G/C), detoxication: GSTM1 (del), GSTT1(del), CYP1A1 (6235 T/C), CYP2E1 (-1019C/T), methylation: (MTHFR (677C/T), COMT (Val158Met), DNMT3b (-149C/T) genes in mothers of healthy and suffering from schizophrenia persons.

158 mothers of patients and 82 mothers with healthy progeny were studied by PCR-RFLP.

**Results:** Significant differences in the distribution of COMT and IL-6 alleles were revealed between two groups. For the mothers of patients val-val/COMT (OR=3.82; rs4680) and gc/IL-6 (OR=3.13; CI: 1.5829 - 6.1825; rs1800795) were proved to be risk genotypes; val/COMT (OR=2.31; CI: 1.4275 to 3.7552) and w1/CYP1A1 (OR=2.27; CI: 1.1515 to 4.4586; rs 4646903) were elucidated as risk alleles.

Also the genotype combinations frequencies of functionally different genes varied significantly in examined maternal groups.

**Conclusions:** These results support the general hypothesis that connects the predisposition to schizophrenia with the anomalies during the prenatal development of nervous system. Possibly, processes of inflammation, detoxication and methylation, biochemically interconnected and determined in the maternal genome, can guide the peculiarities of fetal neurodevelopment that later enhance the risk of mental disorder.

#### P09.133

##### Analysis of exome sequence data provides further support for the involvement of histone pathways in the aetiology of schizophrenia

*D. Curtis<sup>1,2</sup>;*

<sup>1</sup>UCL Genetics Institute, London, United Kingdom, <sup>2</sup>Barts and the London School of Medicine and Dentistry, London, United Kingdom.

Weighted burden pathway analysis was applied to whole exome sequence data for 2045 schizophrenic subjects and 2045 controls. More weight was given to variants which were rare and/or to variants predicted to have a functional effect. The 1454 „all GO gene sets, gene symbols“ pathways were downloaded from the Molecular Signatures Database and were used to define gene sets. Overall, there was a statistically significant excess of pathways with more rare, functional variants in cases than controls. Among these were pathways involved in histone modification, as well neuron differentiation and membrane and vesicle function. This bolsters the evidence from other studies that histone modification pathways may be important in the aetiology of schizophrenia.

#### P09.134

##### First exome sequencing study in multiply affected families with schizophrenia from Indonesia

*F. Degenhardt<sup>1</sup>, A. C. Koller<sup>1</sup>, K. U. Ludwig<sup>1</sup>, S. Heilmann-Heimbach<sup>1</sup>, S. Sivalingam<sup>1</sup>, J. Strohmaier<sup>2</sup>, H. Thiele<sup>3</sup>, P. Nürnberg<sup>3</sup>, M. Rietschel<sup>2</sup>, A. Kusumawardhani<sup>4</sup>, D. Widenauer<sup>5</sup>, S. Schwab<sup>6</sup>, M. M. Nöthen<sup>1</sup>;*

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genetic Epidemiology in Psychiatry at the Central Institute of Mental Health, University Medical Center Mannheim/University of Heidelberg, Mannheim, Germany, <sup>3</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>4</sup>The University of Western Australia, Crawley, Australia, <sup>5</sup>, Perth, Australia.

Schizophrenia (SCZ) is a common neuropsychiatric disorder with an estimated heritability of approximately 60-80%. So far, only a small proportion of its underlying genetic risk factors have been identified. In the search for new risk factors, the analysis of multiply affected families is a very promising approach. Here, we present the first exome sequencing study in multiply affected families with SCZ from Indonesia. In each of the nine families, three to five genetically distant individuals were exome sequenced on an Illumina HiSeq2500. For the data analyses the Varbank pipeline (<http://varbank.ccg.uni-koeln.de>), and the CLC Biomedical Genomics Workbench were used. For the downstream analyses only those variants were taken into account that were predicted to be deleterious, rare in publicly available databases (minor allele frequency <0.1%), experimentally confirmed, and co-segregating within the respective family. On average, we have identified 30-50 mutations per family that fulfil these criteria. Indonesian samples are underrepresented in the publicly available databases. Therefore the most promising variants are genotyped in 1,000 controls from Indonesia to exclude population stratification. All variants with a MAF >0.1% are excluded. Not all genes implicated in our study will be relevant for SCZ. In search for additional genetic evidence for the implicated genes, we check publicly available SCZ exomes (>2,500 index patients and >1,000 parent-proband trios) and additional 150 in-house exomes of severely affected SCZ patients for the presence of mutations in the identified genes. The study is ongoing and more detailed data will be presented at the conference.

#### P09.135

##### Genetic variants in minor physical anomalies and craniofacial measures in schizophrenia

*A. Chen<sup>1</sup>, S. Wang<sup>1</sup>, P. Hsiao<sup>1</sup>, L. Yeh<sup>2</sup>, C. Liu<sup>3</sup>, C. Liu<sup>3</sup>, T. Hwang<sup>3</sup>, M. Hsieh<sup>3</sup>, Y. Chien<sup>3</sup>, Y. Lin<sup>3</sup>, S. D. Chandler<sup>4</sup>, S. J. Glatt<sup>4</sup>, N. Laird<sup>6</sup>, S. V. Faraone<sup>5</sup>, M. Tsuang<sup>4</sup>, H. Hwu<sup>1,3,7</sup>, W. Chen<sup>1,7,8</sup>;*

<sup>1</sup>Institute of Epidemiology and Preventive Medicine, College of Public Health, Taipei, Taiwan, <sup>2</sup>Department of Healthcare Administration, College of Health Science, Asia University, Taichung, Taiwan, <sup>3</sup>Department of Psychiatry, College of Medicine and National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan,

<sup>4</sup>Center for Behavioral Genomics, Department of Psychiatry, University of California San Diego, La Jolla, CA, United States, <sup>5</sup>Departments of Psychiatry and Behavioral Sciences and Neuroscience and Physiology, Medical Genetics Research Center, SUNY Upstate Medical University, Syracuse, NY, United States, <sup>6</sup>Department of Biostatistics, Harvard University, Boston, MA, United States, <sup>7</sup>Institute of Brain and Mind Sciences, College of Medicine, National Taiwan University, Taipei, Taiwan, <sup>8</sup>Genetic Epidemiology Core Laboratory, Division of Genomic Medicine, Research Center for Medical Excellence, National Taiwan University, Taipei, Taiwan.

##### Background

Schizophrenia (SZ) patients and their first-degree relatives show more minor physical anomalies (MPAs)/abnormal craniofacial features (CFs) than the general population. Family studies found moderate heritability of MPAs/CFs, but no genetic markers have been consistently identified. This study aims to 1) examine the relationships between MPAs/CFs and clinical characteristics; and 2) identify MPAs/CFs-related genetic markers in schizophrenia.

##### Methods

A genome-wide association study was performed in 1,123 SZ patients to search for genetic variants of MPAs/CFs. Using a 6-item questionnaire to collect MPAs/CFs measurements and calculate a MPAs score. Genotyping was conducted using the PsychChip microarray co-developed by PGC and illumina. Polygenic risk score analysis was applied to predict individual MPAs score.

##### Results

A total of 1,067 SZ patients and 330,000 SNPs entered final analysis. Patients with more hair whorls ( $p<0.03$ ) or nostrils antverted ( $p<0.01$ ) showed higher positive symptoms scores. Micrognathia was associated with both positive ( $p<0.0003$ ) and negative ( $p<0.0003$ ) symptoms. A polygenic score was significantly associated with patients with higher MPAs score ( $p<0.0001$ ). No single nucleotide polymorphism reached genome-wide significance.

##### Conclusions

Our finding suggests that multiple variants with small effects influence MPAs/CFs in SZ. Further investigation into the pathways or biological functions of these variants would help us understand more about MPAs and its relationship to SZ.

##### Grant references

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#### P09.137

##### Exome sequencing in multiply affected families identifies new candidate genes for schizophrenia

*A. C. Koller<sup>1,2</sup>, K. U. Ludwig<sup>1,2</sup>, S. Heilmann-Heimbach<sup>1,2</sup>, S. Sivalingam<sup>1,2</sup>, J. Strohmaier<sup>3</sup>, J. Hecker<sup>4,1</sup>, M. Lang<sup>3</sup>, H. Löhllein Fier<sup>5,4,1</sup>, A. Maaser<sup>1,2</sup>, A. J. Forstner<sup>1,2</sup>, H. Thiele<sup>6</sup>, P. Nürnberg<sup>6</sup>, T. G. Schulze<sup>7</sup>, M. Rietschel<sup>2</sup>, M. M. Nöthen<sup>1,2</sup>, A. Reif<sup>8</sup>, F. Degenhardt<sup>1,2</sup>;*

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life and Brain Center, Bonn, Germany, <sup>3</sup>Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, University Medical Center Mannheim/University of Heidelberg, Mannheim, Germany, <sup>4</sup>Institute of Genomic Mathematics, University of Bonn, Bonn, Germany, <sup>5</sup>Department of Biostatistics at the Harvard T.H. Chan School of Public Health, Boston, MA, United States, <sup>6</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>7</sup>Institute for Psychiatric Phenomic and Genomic (IPPG), Ludwig-Maximilians-University, Munich, Germany, <sup>8</sup>Department of Psychiatry Psychosomatic Medicine and Psychotherapy, University of Frankfurt, Frankfurt, Germany.

Schizophrenia (SCZ) is a common disorder with a lifetime risk of ~1%. Only few of the patients are additionally diagnosed with cataphasia (speech disorder) or periodic catatonia (movement disorder). To the authors' knowledge, this is the first study performing exome sequencing in patients with these infrequent subphenotypes.

Four genetically distant individuals (from three multiply affected families) were exome sequenced. For the data analyses the Varbank pipeline (<http://varbank.ccg.uni-koeln.de>), and the CLC Biomedical Genomics Workbench were used. The analyses included only those variants that were: (i) predicted to be deleterious (Combined Annotation Dependent Depletion score  $\geq 15$ ; <http://cadd.gs.washington.edu/>), (ii) rare in publicly available databases (minor allele frequency  $\leq 0.1\%$ ), (iii) confirmed by Sanger sequencing,

and (iv) co-segregating within the respective family.

The initial results of the analysis revealed 35 variants in 35 genes. We are currently genotyping all identified variants in 1,000 German population-based controls and will exclude variants with a minor allele frequency  $\geq 0.1\%$ . In addition, we performed gene-based tests (as implemented in VEGAS) in a genome-wide association study of SCZ (35,000 patients, 100,000 controls).

To validate the candidate genes identified, we will: (i) check publically available SCZ datasets ( $>2,500$  exomes) and additional 50 in-house multiply affected SCZ families for the presence of mutations in the identified genes; (ii) re-sequence the most promising genes in independent patients with SCZ and controls. So far, our most promising candidate genes are ZNF426, NOS3, and MECP2. For these, at least one de novo mutation in a patient with SCZ was reported in the literature.

#### P09.138

#### Identification of six novel mutations in SCN1A gene in Hungarian patients with epilepsy

J. Bene<sup>1,2</sup>, K. Hadzsiev<sup>1,2</sup>, T. Szabó<sup>1</sup>, E. Pöstyeni<sup>1</sup>, E. Kovácsdi<sup>1,2</sup>, R. Szalai<sup>1,2</sup>, A. Till<sup>1</sup>, A. Fekete-Locsei<sup>1</sup>, B. Melegi<sup>1,2</sup>;

<sup>1</sup>Department of Medical Genetics, University of Pécs, Pécs, Hungary, <sup>2</sup>Szentagothai Research Centre, Pécs, Hungary.

**Introduction:** Epilepsies are a heterogeneous group of neurological disorders characterized by recurrent and unprovoked seizures. Family and twin studies revealed that 30-40 % of the epilepsy cases have a genetic component. Monogenic form of epilepsy represents 1 % of the idiopathic epilepsies and mutations of ion channel genes play a major role in the pathogenesis of this type of epilepsy. Among the ion channel genes mutations in *SCN1A* (sodium channel type 1 alpha subunit) gene are the most frequent and most clinically relevant. The aim of our study was to characterize the *SCN1A* mutation spectrum in Hungarian epilepsy patients.

**Materials and methods:** We performed the *SCN1A* gene mutation analysis of 106 Hungarian patients with epilepsy phenotype using Sanger sequencing.

**Results:** A total of 12 different point mutations were identified. Half of the mutations were newly identified in 9 patients and involve 4 (67%) missense and 2 (33%) frameshift mutations. Moreover we detected 6 different known pathogenic *SCN1A* mutations in 10 patients, in which 3 (30.0%) of them were missense mutation, 2 (20.0%) of them were nonsense mutation, also 2 (20.0%) of them were splice region variant, and one (10.0%) of them was a frameshift-causing deletion.

**Conclusions:** The *SCN1A* gene mutation analysis of Hungarian patients with epilepsy resulted in the identification of 6 novel mutations, which could expand the spectrum of *SCN1A* mutations and supports the current understanding of genotype-phenotype correlations.

#### P09.139

#### Rare compound heterozygous variants in SCN3A in a patient with congenital hypotonia, micrognathia, trismus and developmental delay

I. L. Mero<sup>1</sup>, A. M. Øye<sup>1</sup>, Y. Sheng<sup>1</sup>, S. C. Wallace<sup>2</sup>, T. Stiris<sup>3</sup>, H. E. Høgevold<sup>4</sup>, B. Paus<sup>5</sup>, M. D. Vigeland<sup>1</sup>, K. K. Selmer<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Department of Pediatrics, Oslo University Hospital, Oslo, Norway, <sup>3</sup>Neonatal Intensive Care Unit, Oslo University Hospital, Oslo, Norway, <sup>4</sup>Craniofacial team and Department of Plastic and Reconstructive Surgery, Oslo University Hospital, Oslo, Norway, <sup>5</sup>Department of Medical Genetics, Oslo University Hospital and Institute of Clinical Medicine University of Oslo, Oslo, Norway.

We report on a girl born to healthy non-consanguineous parents who previously experienced a stillbirth due to an umbilical cord knot. The patient was born at 38 weeks gestation, birth weight 2.7 kg. She presented with pronounced hypotonia, reduced suck-swallow reflex, micrognathia and trismus. At 24 months she has global developmental delay. She is only able to open her mouth 1cm, however during sedation full-range movement of her jaw is obtained. There is no history of seizures. Brain MRIs were initially reported normal, whereas a recent MRI shows volume loss of the cerebral peduncles, hypoplastic corpus callosum and increased T2/FLAIR signaling in the thalamic regions. The findings are suspicious of periventricular leucomalacia, but the clinical presentation does not support this diagnosis.

Trio exome sequencing identified two missense variants in NM\_001081676 (*SCN3A*): c.5278G>A (p.E1760K) and c.A154A>C (p.N52H), each inherited from one parent. The former variant has a frequency of 5.77e-05 in ExAC and the latter has never been observed in ExAC, 1000GP, ESP6500, nor in our inhouse Norwegian database. Both variants are highly conserved (GERP 6.17 and 5.32 respectively), predicted pathogenic by SIFT and PolyPhen2 and CADD-scores are 16.74 and 23.2.

*SCN3A* is a sodium channel predominantly expressed in brain and muscle,

and not previously reported to cause autosomal recessive disease. Heterozygous mutations have been linked to focal epilepsy of childhood and in one case to encephalopathy with multiple congenital anomalies. Additional cases are needed to confirm whether mutations in *SCN3A* could cause an autosomal recessive neurodevelopmental syndrome.

#### P09.140

#### A French collaborative study of genotype - phenotype relationships in 78 patients carried a 22q13.3 genomic imbalance

A. Tabet<sup>1,2</sup>, M. Duclouy<sup>3</sup>, J. Buratti<sup>3</sup>, A. Mathieu<sup>3</sup>, B. Keren<sup>4</sup>, E. Pipiras<sup>5</sup>, S. Chantot-Bastaraud<sup>6</sup>, C. Le Caignec<sup>7</sup>, J. Dupont<sup>8</sup>, S. Kemeny<sup>9</sup>, A. Mosca Boidron<sup>10</sup>, C. Missirian<sup>11</sup>, D. Sanlaville<sup>12</sup>, G. Vieville<sup>13</sup>, M. Vuillaume<sup>14</sup>, R. Delorme<sup>3,15</sup>, T. Bourgeron<sup>3</sup>;

<sup>1</sup>Robert Debre Hospital APHP, paris, France, <sup>2</sup>GHFC unit, Pasteur institute, Paris, France, <sup>3</sup>GHFC unit, Pasteur institute, paris, France, <sup>4</sup>Genetic unit, Pitié Salpêtrière hospital, paris, France, <sup>5</sup>Cytogenetic unit, Jean Verdier Hospital, Bondy, France, <sup>6</sup>Cytogenetic Unit, Troussseau Hospital, paris, France, <sup>7</sup>Genetic Unit, Nantes Hospital, Nantes, France, <sup>8</sup>Cochin Hospital APHP, paris, France, <sup>9</sup>Genetic Unit, CHU Estaing, Clermont Ferrand, France, <sup>10</sup>Cytogenetic Unit, Dijon Hospital, Dijon, France, <sup>11</sup>Genetic Unit, La Timone Hospital, Marseille, France, <sup>12</sup>Lyon Civil Hospital, Lyon, France, <sup>13</sup>Cytogenetic Unit, Grenoble Hospital, Grenoble, France, <sup>14</sup>Genetic unit, Bordeaux hospital, Bordeaux, France, <sup>15</sup>Psychopathologic Unit, Robert Debre Hospital, Paris, France.

Patients with Phelan McDermid Syndrome, (or 22q13.3 deletion syndrome) often have intellectual disability (ID), language impairment and autism. SHANK3 is one of the genes that contribute to the phenotype. There is a high clinical heterogeneity and the genotype-phenotype correlation is still unclear. Our French collaborative study aims to precise it and look for potential modifying genetic factors.

We reported genetic and phenotypic data from 78 patients, 73 carried a 22q13.3 deletion and 5 had a duplication ranging in size from 45.8 kb to 9.10 Mb.

The neonatal period revealed mild motor developmental delay in deleted carriers. All patients exhibited language disorder and 50% had no verbal communication. Autistic traits were reported in 50% of cases, unfortunately, objective tests were available only for three patients. Most patients had intellectual deficiency and medical comorbidity were also noted. Long-term follow up of two patients showed motor regression and acute psychiatric disorder episode at adulthood. Patients with duplication had language disturbance and three presented with autistic traits.

We studied the correlation between the size of the deleted segments and the main clinical features. We also analyzed the presence of other CNVs affecting gene coding sequences to detect potential "second hit" implicated in the clinical heterogeneity of the syndrome.

Interestingly, 2 patients with deletion had also a CNV at 16p11.2 involved in ASD, ID and obesity.

Based on the results of this pilot study, we aim to extend this research to other European and International centers to identify factors that modulate the severity of the disorder.

#### P09.141

#### Heterozygous HTRA1 mutations are associated with autosomal dominant cerebral small vessel disease

E. Verdura<sup>1,2</sup>, D. Hervé<sup>1,2,3</sup>, E. Scharrer<sup>4</sup>, M. Amador<sup>5</sup>, L. Guyant-Maréchal<sup>6</sup>, A. Philippi<sup>1,2</sup>, A. Corlobé<sup>7</sup>, F. Bergametti<sup>1,2</sup>, S. Gazal<sup>8</sup>, C. Prieto-Morin<sup>1,2,5</sup>, N. Beaufort<sup>4</sup>, B. Le Bail<sup>9</sup>, I. Viakhireva<sup>10</sup>, M. Dichgans<sup>4,11</sup>, H. Chabriat<sup>1,2,3</sup>, C. Haffner<sup>4</sup>, E. Tournier-Lasserre<sup>1,2,5</sup>,

<sup>1</sup>INSERM UMR-1161, Génétique et Physiopathologie des Maladies Cérébro-vasculaires, Paris, France, <sup>2</sup>Université Paris Diderot, Sorbonne Paris Cité (SPC), Paris, France, <sup>3</sup>AP-HP, Groupe Hospitalier Saint-Louis Lariboisière-Fernand-Widal, Service de

Neurologie, Centre de Référence des Maladies Vasculaires Rares du Cerveau et de l'Œil (CERVCO), Paris, France, <sup>4</sup>Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig Maximilians University, Munich, Germany, <sup>5</sup>AP-HP, Groupe Hospitalier Saint-Louis Lariboisière-Fernand-Widal, Service de Génétique Moléculaire Neurovasculaire, Centre de Référence des Maladies Vasculaires Rares du Cerveau et de l'Œil (CERVCO), Paris, France, <sup>6</sup>Service de Neurophysiologie, Hôpital Charles Nicolle, Rouen, France, Rouen, France, <sup>7</sup>Service de Neurologie, Hôpital Gui de Chauliac, Montpellier, France, <sup>8</sup>Plateforme de Génomique Constitutionnelle du GHU Nord, Assistance Publique des Hôpitaux de Paris (APHP), Hôpital Bichat, Paris, France, <sup>9</sup>Service de Neurologie, CH Bretagne Sud, Lorient, France, <sup>10</sup>Service de Neurologie, Hôpital de la Cavale Blanche, Brest, France, <sup>11</sup>Munich Cluster for Systems Neurology (SyNergy), Munich, Germany, Munich, Germany.

**Introduction:** Cerebral Small Vessel Disease (SVD) represents a heterogeneous group of disorders leading to stroke and cognitive impairment. Various monogenic forms have been reported. However, they only account for a minority of familial SVD patients.

**Material and methods:** Pangenomic linkage analysis and whole exome sequencing were used to identify candidate genes in an autosomal dominant SVD family for which known SVD genes had been excluded. Candidate genes were then screened in 201 unrelated probands with a familial SVD of

unknown etiology.

Results: A heterozygous variant within HTRA1 (Arg166Leu), absent from public databases and predicted to be deleterious by *in silico* tools was identified in all affected members of the index family. Ten probands out of the 201 unrelated familial SVD probands (4.97 %) harbored a heterozygous HTRA1 mutation predicted to be damaging. There was a highly significant difference in the number of likely deleterious variants in cases compared to controls ( $p=4.2\times 10^{-6}$ ; OR=15.4; CI=4.9-45.5), strongly suggesting causality. *In vitro* activity analysis of HTRA1 mutants demonstrated a loss-of-function effect. Clinical and MRI features of this autosomal dominant SVD differed from those of CARASIL (a very rare, autosomal recessive SVD form, caused by biallelic HTRA1 mutations) by a later age of onset and the absence of extraneurological features.

Conclusions: Our data demonstrate that heterozygous HTRA1 mutations are an important cause of familial SVD. Screening of HTRA1 should be considered in hereditary SVD patients. Investigation of the pathogenicity of these heterozygous mutations in the trimeric HtrA1 enzyme is ongoing.

#### P09.142

#### Compound heterozygous SPATA5 mutations cause progressive neurodegenerative disorder with microcephaly, seizures and hearing loss

**K. Ounap<sup>1,2</sup>, S. Pajusalu<sup>1,3</sup>, K. Reinson<sup>1,2</sup>, A. Kaasik<sup>4</sup>, I. Talvik<sup>5</sup>, Ü. Murumets<sup>1</sup>, T. Reimand<sup>1,2,3</sup>,**

<sup>1</sup>Department of Genetics, Tartu University Hospital, Tartu, Estonia, <sup>2</sup>Department of Paediatrics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia,

<sup>3</sup>Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, <sup>4</sup>Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, <sup>5</sup>Tallinn Children's Hospital, Tallinn, Estonia.

Tanaka et al. (2015) recently identified 14 individuals with microcephaly, developmental delay, intellectual disability, hypotonia, spasticity, seizures, sensorineural hearing loss, cortical visual impairment, and biallelic presumably disease causing variants in spermatogenesis-associated protein 5 (SPATA5). SPATA5 encodes a ubiquitously expressed member of the ATPase protein family which is involved in mitochondrial morphogenesis during early spermatogenesis. Here we report two siblings with similar phenotype and compound heterozygous mutations in the SPATA5 gene.

Index patient had developmental delay since 2 months of age and remarkable regression since 7 months. At 1 y she had microcephaly (-2 SD), spasticity, dystonic movements, strabismus, no eye contact, sensorineural hearing loss and tonic-clonic seizures. Brain MRI showed moderate cerebral atrophy, a slightly elevated signal intensity in white matter, and atrophic caudate nucleus suggesting a neurodegenerative disorder. Lysosomal disorders have been excluded by enzyme analyses. Elder brother had similar progressive disease and both died at 3 years of age.

Whole exome sequencing in index case identified two rare heterozygous mutations in SPATA5 gene: c.250C>T p.Arg84\* and c.989\_991del p.Thr330del (NM\_145207.2). Compound heterozygosity of both affected siblings and heterozygosity of only c.250C>T in the mother was confirmed by Sanger sequencing.

The described family supports the conclusion by Tanaka et al. that mutations in SPATA5 might affect brain development and function, resulting in microcephaly, developmental delay, and intellectual disability. We are currently performing functional studies on patients' fibroblasts to further assess the role of the SPATA5 mutations.

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#### P09.143

#### Multidisciplinary investigation of backward-speech trait suggests a link between RIC3, RIPK1, ZBED5 and working memory

**S. Preković<sup>1</sup>, D. Filipović Đurđević<sup>2,3</sup>, G. Csifcsák<sup>4</sup>, O. Sveljo<sup>5,6</sup>, O. Stojković<sup>7</sup>, F. Ceroni<sup>8</sup>, D. F. Newbury<sup>9</sup>,**

<sup>1</sup>Faculty of Medicine, KU Leuven, Leuven, Belgium, <sup>2</sup>Faculty of Philosophy, University of Novi Sad, Novi Sad, Serbia, <sup>3</sup>Faculty of Philosophy, University of Belgrade, Belgrade, Serbia, <sup>4</sup>Institute of Psychology, Faculty of Arts, University of Szeged, Szeged, Hungary,

<sup>5</sup>Diagnostic Imaging Center, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia,

<sup>6</sup>Faculty of Technical Sciences, University of Novi Sad, Novi Sad, Serbia, <sup>7</sup>Faculty of Medicine, University of Belgrade, Belgrade, Serbia, <sup>8</sup>Wellcome Trust Center for Human Genetics, University of Oxford, Oxford, United Kingdom.

Introduction: Working memory is essential for the development of many language-related traits. It has been suggested that the rare trait of backward-speaking is linked to working memory. This trait is described as an ability to spontaneously and accurately reverse words. Here we describe individuals (the father and daughter) from a Serbian family who have the

ability to speak backward voluntarily.

Materials and Methods: We employed behavioral tests to describe the trait and neuroimaging (EEG and fMRI) to study the neural processing behind backward-speech. Moreover, we investigated coding sequence changes through exome sequencing and copy number variations using SNP array data in this family.

Results: Behavioral data suggests that backward-speech loads heavily upon working memory. Event-related potentials above the frontal lobe are affected by word reversal and the maintenance of backward-words in working memory. fMRI revealed that the left fusiform gyrus may facilitate backward-speech in the daughter. Exome sequencing identified three novel coding variants of potential significance in the RIC3, RIPK1 and ZBED5 genes.

Conclusions: Our data suggest that in the daughter, backward-speech is afforded by an extraordinary working memory capacity. We hypothesize that this is served by cholinergic projections from the basal forebrain to the frontal cortex and supported by visual semantic loops within the left fusiform gyrus and that these processes may be mediated by a genetic mutation in the RIC3 gene which encodes a chaperone for nicotinic acetylcholine receptors.

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#### P09.144

#### Spinal lipoma as a dysembryogenetic anomaly: 4 unusual cases of ectopic iliac rib within the spinal lipoma

**A. Accogli<sup>1</sup>, M. Pavanello<sup>1</sup>, P. Accorsi<sup>2</sup>, P. De Marco<sup>1</sup>, E. Merello<sup>1</sup>, P. Nozza<sup>1</sup>, C. Fiorillo<sup>1</sup>, L. Pinelli<sup>1</sup>, A. Cama<sup>1</sup>, A. Rossi<sup>1</sup>, M. Catala<sup>4,5,6</sup>, V. Capra<sup>1</sup>,**

<sup>1</sup>Istituto Giannina Gaslini, Genova, Italy, <sup>2</sup>U.O. Neuropsichiatria dell'Infanzia e dell'Adolescenza, Spedali Civili, Brescia, Italy, <sup>3</sup>U.O.C. Neuroradiologia, Spedali Civili, Brescia, Italy, <sup>4</sup>Fédération de Neurologie, Groupe hospitalier Pitié-Salpêtrière APHP, Paris, France, <sup>5</sup>Sorbonne Universités, UPMC Université Paris 06, Paris, France, <sup>6</sup>CNRS, Institut de Biologie Paris Seine (IBPS) – Developmental Biology Laboratory, Paris, France.

Background: Congenital spinal lipomas are closed spinal dysraphisms belonging to the Neural Tube Defects (NTDs) group. They include a broad spectrum of lesions ranging from simple lipomas of the filum terminale to complex malformations. On histological evaluation, various tissue components of ectodermal, mesodermal or endodermal origin are found within the lipomas, with prevalence for nerves and striated muscle. Overall, rib malformations have been occasionally observed in patients with NTDs and in NTD mouse models. However, an ectopic rib arising within the spinal lipoma and articulating with the iliac crest has not been reported in either of them.

Cases: We describe four patients affected by lipomyeloschisis or lipomyelomeningocele, with an unusual fibrocartilaginous protuberance arising within the lipoma and connecting to one iliac crest, resembling an ectopic rib. Histological evaluation confirmed the presence of cartilaginous tissue.

Discussion: This anatomical feature is reminiscent of the so-called caudal appendages that can arise elsewhere from the midline. In our cases, the presence of the extra-rib in the sacral region suggests a defect of the genetic pathway that controls the specification of the antero-posterior identity.

Conclusions: We expand the clinical spectrum of fibrocartilaginous anomalies associated with spinal lipoma, suggesting the presence of an ectopic rib as a new possible phenotype in NTDs. A careful analysis by neuroradiologists and pathologists should be performed in spinal lipomas to assess the presence of an ectopic rib or other uncommon developmental anomalies. Furthermore, molecular studies are required to detect the genetic cause of this unusual phenotype.

#### P09.145

#### In-vitro characterization of STUB1 mutations in recessively inherited spinocerebellar ataxia-16

**Y. Pakdaman<sup>1,2</sup>, M. Sanchez-Guixé<sup>1,2</sup>, R. Kleppe<sup>3,4</sup>, K. Heimdal<sup>5</sup>, P. M. Knappskog<sup>1,2</sup>, I. Aukrust<sup>6</sup>, S. Johansson<sup>1,2</sup>,**

<sup>1</sup>Department of Clinical Science, University of Bergen, Bergen, Norway, <sup>2</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, <sup>3</sup>Department of Biomedicine, University of Bergen, Bergen, Norway, <sup>4</sup>K.G. Jebsen Centre for Neuropsychiatric Disorders, University of Bergen, Bergen, Norway,

<sup>5</sup>Department of Medical Genetics, Oslo University Hospital, Oslo, Norway.

Introduction: Autosomal recessive spinocerebellar ataxia-16 is caused by mutations in *STUB1* encoding the ubiquitin E3 ligase and dimeric co-chaperone CHIP. There is limited knowledge about the mechanisms whereby these mutations cause the disease. This study aims to characterize the structure and function of six previously published pathogenic CHIP mutations.

Material and methods: Six mutations E28K, N65S, K145Q, M211I, S236T, and T246M were made by site-directed mutagenesis, and purified as recom-

binant proteins from *E. coli*. The mutants were analyzed and compared with wild-type CHIP by ubiquitination activity assay, limited proteolysis assay, oligomerization analysis by gel-filtration chromatography, and circular dichroism.

Results: Only N65S and T246M showed impaired ability to ubiquitinate the HSC70 chaperone. The limited proteolysis assay showed an increased stability against trypsin digestion for N65S, while T246M and E28K were more rapidly cleaved than wild-type, indicating a less compact protein structure. By gel-filtration chromatography analysis N65S showed a sharp dimeric peak compared to T246 and E28K that both generated broad oligomeric peaks. K145Q, M211I and S236T behaved overall similar to wild-type CHIP. Further characterization is currently being performed using circular dichroism to analyze protein structure/stability, and in cellular systems to analyze subcellular localization.

Conclusion: Our results illustrate that some mutations known to cause recessive spinocerebellar atrophy-16 can affect protein structure and ability of CHIP to dimerize *in vitro*. Hence, we speculate that *STUB1* mutations might not only affect CHIP's E3 ubiquitin ligase properties and interaction with its chaperones, but may also affect its protein structure and possibly stability *in vivo*.

#### P09.146

#### Syntaptotagmin-1 mutation is a recurrent disorder of neurotransmitter release

**K. Baker**<sup>1</sup>, S. L. Gordon<sup>2</sup>, UK10K Consortium, M. A. Cousin<sup>3</sup>, F. L. Raymond<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom,

<sup>2</sup>The Florey Institute, Melbourne, Australia, <sup>3</sup>Centre for Integrative Physiology, University of Edinburgh, Edinburgh, United Kingdom.

Syntaptotagmin-1 (*SYT1*) is a calcium-binding synaptic vesicle protein required for both exocytosis and endocytosis. We recently described the first human condition associated with a rare variant in *SYT1*. The individual harbouring this variant presented with an early onset dyskinetic movement disorder, severe motor delay, and profound cognitive impairment. Structural MRI was normal, but EEG showed extensive neurophysiological disturbances. Trio analysis of whole-exome sequence identified a de novo *SYT1* missense variant (I368T). Expression of rat *SYT1* containing the equivalent human variant in WT mouse primary hippocampal cultures revealed that the mutant form of *SYT1* correctly localizes to nerve terminals and is expressed at levels that are approximately equal to levels of endogenous WT protein. The presence of the mutant *SYT1* slowed synaptic vesicle fusion kinetics, a finding that agrees with the previously demonstrated role for I368 in calcium dependent membrane penetration. Expression of the I368T variant also altered the kinetics of synaptic vesicle endocytosis. Subsequently, three further cases of neurodevelopmental disorder associated with de novo mutation in *SYT1* have been identified. In common with the first case, structural brain development is normal whilst cortical electrophysiology is severely disturbed, though no case has experienced overt seizures. Motor development is consistently less severely disrupted than communication and social development. Unpredictable deteriorations in behavioural function are reported in all cases. Together, the clinical features, electrophysiological phenotype, and *in vitro* neuronal phenotype associated with *SYT1* mutation highlight presynaptic mechanisms of synchronous neurotransmitter release and short term plasticity that mediate human motor control and cognitive development.

#### P09.147

#### Behavioral analysis of mouse model with combined deficiencies of $\beta$ -Hexosaminidase A and sialidase Neu3 reveals motor coordination impairment and memory loss

**S. Akyildiz Demir**<sup>1</sup>, E. Erdemli<sup>2</sup>, B. Demir<sup>3</sup>, T. Dalkara<sup>3</sup>, T. Miyagi<sup>4</sup>, V. Seyrantepe<sup>1</sup>;

<sup>1</sup>Izmir Institute of Technology, Izmir, Turkey, <sup>2</sup>Ankara University, Faculty of Medicine, Ankara, Turkey, <sup>3</sup>Hacettepe University, Ankara, Turkey, <sup>4</sup>Tohoku Pharmaceutical University, Miyagi, Japan.

Tay-Sachs disease is a severe lysosomal storage disorder caused by mutations in the HEXA gene coding for  $\alpha$  subunit of lysosomal  $\beta$ -Hexosaminidase A enzyme, which converts GM2 to GM3 ganglioside. Unexpectedly, the HexA-/- mice have a normal lifespan and show no obvious neurological impairments until at least 1 year of age, owing to the ability of these mice to catabolise stored GM2 ganglioside via sialidase Neu3 removing sialic acid into glycolipid GA2 which further processed by  $\beta$ -Hexosaminidase B, thereby bypassing the HexA defect. To elucidate whether sialidase Neu3 can contribute to GM2 ganglioside degradation, we generated mice model with combined deficiencies of  $\beta$ -Hexosaminidase A and sialidase Neu3. HexA-/-Neu3-/- mice are

healthy at birth but died at 1.5-4.5 months of age. Slow movement, ataxia and tremor are among neurological abnormalities. TLC and IHC analysis showed massive accumulation of GM2 ganglioside in brain. Electron and light microscopy analysis of brain cortex showed lysosomal storage disease pathology similar to Tay-Sachs patient's. Behavioral analysis including passive-avoidance, rotarod and water maize revealed age-dependent motor coordination impairment and memory deficit which indicate progressive neurodegeneration in HexA-/-Neu3-/- mice compared to HexA-/. The unexpected severe phenotype of HexA-/- mice appeared to be influenced by the status of sialidase Neu3 gene.

#### P09.148

#### Genetic variants of TREM2 associated with neurodegenerative disorders

**B. J. Peplonska**, M. Berdynski, M. Kobrynski, C. Zekanowski;

Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland.

Introduction: TREM2 encodes triggering receptor expressed on myeloid cells 2 expressed on the cell membrane of many types of immune cells including macrophages, dendritic cells, osteoclasts and microglia. Activation of the TREM2 receptor on microglia stimulates phagocytosis activity and decreases microglial proinflammatory responses. The protein may play a role in cleaning damaged or apoptotic cells and cellular debris and help resolve damage-induced inflammation. Given the reported antiinflammatory role of TREM2 in the brain, mutations and polymorphic variants may increase the risk of neurodegenerative conditions such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD). Materials and Methods: The study group consisted of: 250 neurologically normal controls, 150 AD, 192 ALS, 144 FTD patients. TREM2 exon 2 with flanking intronic sequences was mined using Sanger fluorescent method (ABI 3130) in the whole studied group.

Results: Six rare variants located in exon 2 of TREM2 were identified, predominantly in heterozygous state. Three of them (R62H, R62C, D87N) are present in all groups. Variant D87N in homozygous state was identified in one FTD patient. R98W was found only in one AD patient, T66T and T66M were identified in FTD group only. The results suggest that variant T66M is in linkage disequilibrium with R62C one.

Conclusion: Due to modulatory action of TREM2 on inflammatory immune responses in microglia, we suggest that mutations and polymorphisms in TREM2 could contribute to disease pathogenesis in the Polish population.

#### P09.149

#### Synaptic plasticity and cognitive function are disrupted in a mouse model of Williams-Beuren syndrome

**C. Borralleras**<sup>1,2,3</sup>, T. Amédée<sup>4</sup>, S. Mato<sup>5</sup>, G. Albericio<sup>2</sup>, C. Mulle<sup>4</sup>, L. A. Pérez-Jurado<sup>1,2,3</sup>, V. Campuzano<sup>1,2,3</sup>;

<sup>1</sup>Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain,

<sup>2</sup>Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), IISICII, Barcelona, Spain, <sup>4</sup>Institut Interdisciplinaire de Neurosciences, CNRS UMR 5297 Université de Bordeaux 2, Bordeaux, France, <sup>5</sup>Department of Neuroscience, Neurotek-University of the Basque Country, Leioa, Spain.

Introduction: Williams-Beuren Syndrome is a rare neurodevelopmental disorder caused by a heterozygous deletion of 26-28 genes on chromosome band 7q11.23 manifesting a characteristic cognitive and behavioral profile including intellectual disability, increased general anxiety, overfriendly personality and visuospatial deficits. The complete deletion (CD) mouse model, which carries the same deletion found in WBS patients, recapitulates relevant features of WBS neurocognitive phenotype and shows a reduction in spine density in CA1 hippocampus. However, the mechanisms underlying these phenotypes are still unknown.

Materials and methods: We used some behavioral paradigms related to memory function to study cognitive dysfunction in CD mice. We also investigated synaptic transmission and plasticity by recording excitatory postsynaptic currents (EPSCs) at hippocampal CA3-CA1 glutamatergic synapses. Finally, the expression of molecules associated with synaptic plasticity was characterized by immunofluorescence techniques.

Results: Behavioral characterization of CD mice showed cognitive dysfunction, specifically in spatial working memory. In addition, some alterations in synaptic transmission and plasticity were present in CA1 hippocampus of CD mice, including a significantly reduced long-term potentiation. We also observed molecular dysregulation of some synaptic plasticity markers such as a reduction in BDNF expression in pyramidal layer of CA1 in CD mice.

Conclusions: Taken together, these data suggest that behavioral and cognitive deficits of CD mice could be linked to alterations in glutamatergic synaptic transmission and plasticity in hippocampus. In addition, our results

identify dysregulation of some synaptic plasticity markers that could be potential targets for a pharmacological therapy.

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#### P09.150

#### Effects of GTF2I-GTF2IRD2 interaction on the Williams-Beuren syndrome cognitive phenotype

G. Albericio<sup>1</sup>, A. Antonell<sup>2</sup>, C. Borralleras<sup>3,4</sup>, D. Pérez<sup>1,3,4</sup>, L. A. Pérez Jurado<sup>1,3,4</sup>, V. Campuzano<sup>1,3,4</sup>,

<sup>1</sup>Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Neurology Service, Hospital Clínic, Barcelona, Spain, <sup>3</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain.

**Introduction:** Williams-Beuren syndrome (WBS) is a rare neurodevelopmental disorder caused by a heterozygous deletion at chromosome band 7q11.23. Haploinsufficiency for two General Transcription Factor genes (GTF2I coding for TFII-I and GTF2IRD1 coding for BEN-GTF3) has been associated with the WBS cognitive profile. A multi-copy and variably deleted gene of the family, GTF2IRD2, antagonizes TFII-I's activity and may modulate executive function and cognition in WBS. Depending on deletion breakpoints, WBS patients express different GTF2IRD2 copy-number and isoforms, including a chimeric form.

**Methods:** We analyzed by immunofluorescence assays the subcellular localization of TFII-I in neuronal cultures of mice with the complete WBS-deletion (CD). The subcellular localization and interaction between different GTF2IRD2 isoforms and TFII-I was also studied by immunofluorescence and pull-down assays in COS7 cells.

**Results:** We observed a clear reduction of nuclear TFII-I in neurons of CD mice, consistent with gene dosage. GTF2IRD2 isoforms were differentially localized in the cells but all interacted with TFII-I. Transient expression of patient-derived chimeric GTF2IRD2 in neurons of CD mice resulted in an increased amount of nuclear TFII-I.

**Conclusions:** Our data show that interactions between GTF2IRD2 isoforms and TFII-I could affect the nuclear bioavailability of TFII-I and may explain part of the phenotypic variability in WBS. Considering the capital role of TFII-I dosage on WBS sociability and cognition, understanding these mechanisms might provide novel therapeutic targets for these features.

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#### P09.151

#### Association of serotonin transporter gene polymorphisms (5-HTTLPR and rs 25531) and life-histories with psychopathology vulnerability profiles: a study performed with healthy young adults

M. Alvarez<sup>1,2</sup>, P. Pereira<sup>2</sup>, L. Manco<sup>1,2</sup>, S. Cherpé<sup>2</sup>, M. Cunha<sup>3</sup>, J. Pinto Gouveia<sup>4,5</sup>, P. G. Mota<sup>1</sup>,

<sup>1</sup>Department of Life Sciences, University of Coimbra, Coimbra, Portugal, <sup>2</sup>Research Center for Anthropology and Health, University of Coimbra, Coimbra, Portugal, <sup>3</sup>CINEICC, University of Coimbra, Coimbra, Portugal, <sup>4</sup>Faculty of Psychology and Education Sciences, University of Coimbra, Coimbra, Portugal.

**Introduction:** Variation in both 5-HTTLPR and rs25531 polymorphisms, at the serotonin transporter gene SLC6A4, is hypothesized to moderate the response to stress on depression by serotonin transporter levels and/or by amygdala structure. The aim of this study was to look for statistical interactions between genotypes, defined by both polymorphisms, and phenotypes, defined by levels of anxiety, depression and stress, under specific environmental risk factors in young adults.

**Materials and Methods:** A total of 300 Portuguese healthy young adults were assessed for: i) genotype at both 5HTTLPR and rs25531 segregation sites; ii) levels of anxiety, depression and stress estimated by EADS-21 scale; and iii) psychosocial environment stressors, including parents mental illness, childhood abuse and general well-being, using the CTQ and MHC scales, respectively. Statistical interactions between genotype, phenotype and the environmental variables were screened using Generalized Linear Models test (GLM), implemented in IBM SPSS 22.

**Results:** The allele's frequency spectrum found in Portuguese young adults is similar to those observed in other European population samples, and match the values predicted by Hardy-Weinberg equilibrium. Highly significant statistical interactions were found between SLC6A4 low transcriptional genotypes and levels of anxiety, depression and stress scored by EADS-21. Parents depression was the strongest environmental stressor for the development of mood symptoms ( $p < 0.001$ ). Gender had not a significant effect ( $p = 0.077$ ).

**Conclusions:** Our results confirmed the association of 5HTTLPR S and rs25531g alleles to mood symptoms and the usefulness of the interaction gene x environment experimental design in the interpretation of psychopathology vulnerability profiles.

#### P09.152

#### Psychological consequences of pathway alterations as a target for genomic psychology

M. A. Zelenova<sup>1,2,3</sup>, S. G. Vorsanova<sup>2,1,3</sup>, Y. B. Yurov<sup>1,2,3</sup>, A. M. Kazmin<sup>3</sup>, I. Y. Iourov<sup>1,2,4</sup>,

<sup>1</sup>Mental Health Research Center, Moscow, Russian Federation, <sup>2</sup>Research and Clinical Institute for Pediatrics named after YE Veltishev at the Pirogov Russian National Research Medical University, Ministry of Health, Moscow, Russian Federation, <sup>3</sup>Moscow State University of Psychology and Education, Moscow, Russian Federation, <sup>4</sup>Department of Medical Genetics, Russian Medical Academy of Postgraduate Education, Ministry of Health, Moscow, Russian Federation.

**Introduction.** Genomic alterations to molecular pathways influence intellectual functioning. However, psychological consequences of genomic changes remain largely obscure. To get further insights into the emerging field of genomic psychology, new tools for correlating genomic and psychological data appear to be required. Here we propose an approach combining psychological evaluation using International Classification of Functioning (ICF-CY), chromosomal microarray and original bioinformatic approach for network based-classification.

**Materials and methods.** To test the approach, we studied 7 children (16 years) with varying degrees of intellectual disability, autism and congenital malformations. From the ICF-CY, we focused on general mental functions, communication and involvement.

**Results.** Three cases demonstrated copy number variations and losses of heterozygosity spanning genes involved in metabolic pathways (KEGG: hsa01100): PON1, PON2, PON3, UROC1, UGT1A8, UGT1A10. Bioinformatic analysis showed these genomic and epigenomic variations to underlie phenotypic features. Application of ICF-CY showed that all children had little to no phrasal speech (d1331), troubles maintaining cyclic interaction with a person (d3503) and about 50% success in responding to complex requests (more than 3 parameters) (d3102). All children were engaging in symbolic play and shared activities with adults.

**Conclusions.** The correlation between psychological and genomic data can shed light on molecular pathways to brain malfunctioning. Here, we demonstrated that an opportunity to obtain such correlation combining genomic, bioinformatic and psychological methodology does exist. In order to confirm the found typical features and the effectiveness of the approach it should be applied towards a larger cohort. Supported by the Russian Science Foundation (grant:14-15-00411).

#### P10 Neuromuscular disorders

##### P10.01

#### A novel mutation in SLC16A2 gene in a Turkish boy with Allan-Herndon-Dudley Syndrome

E. Tasdelen<sup>1</sup>, V. Bas<sup>2</sup>, H. Aslan<sup>1</sup>, O. Cilingir<sup>1</sup>, E. Erzurumluoglu<sup>1</sup>, M. Ozdemir<sup>1</sup>, B. Durak Aras<sup>1</sup>, O. Kirkbiyik<sup>3</sup>, Y. Kutbay<sup>2</sup>, S. Artan<sup>1</sup>,

<sup>1</sup>Eskişehir Osmangazi University, Faculty of Medicine, Departments of Medical Genetic, Eskişehir, Turkey, <sup>2</sup>Eskişehir State Hospital, Departments of Pediatrics Endocrinology, Eskişehir, Turkey, <sup>3</sup>Tepecik Training and Research Hospital Department of Medical Genetics, Izmir, Turkey.

Allan-Herndon-Dudley syndrome (AHDS, MIM 300523) is an X-linked recessive neurodegenerative disorder, with severe mental retardation, an impaired speech ability, truncal hypotonia, poor head control, generalized muscle weakness, spastic quadriplegia, joint contractures, movement abnormalities and feeding difficulties in combination with changed thyroid hormone (TH) levels. The cases, have increased serum levels of  $FT_3$ , while TSH concentrations are normal and low-normal  $FT4$ . Seizures, poor weight gain, hyperactive deep tendon reflexes observed in some patients. MCT8 is transporter triiodothyronine ( $T_3$ ) hormone, and plays an important role to uptake into neuronal cells. Mutations in the *MCT8*, also known as *SLC16A2*, have been related with AHDS.

We here identified a five years old boy with developmental delay, truncal hypotonia, spastic paraparesis, psychomotor retardation, mild dysmorphic facial features and patognomonic thyroid function tests, such as elevated  $FT3$  and low  $FT4$  levels. Elongated face, poor feeding, pectus carinatum, inability to walk and independently sit, myoclonic movements, hyperreflexia were noted. Thyroid profile and clinical findings of patient that were consistent with AHDS. Sanger sequencing of the *SLC16A2* gene revealed a hemizygous mutation in exon 1 (p.E9X; c.25G>T). The proband's mother and grandmother have heterozygous c.25G>T mutation, but the proband's uncle with psychomotor retardation, could not be analysed, since he had passed away nine months ago. To our knowledge, this mutation has not been previously reported in the etiology of AHDS. Due to generated premature stop codon, the mutation is evaluated disease causing mutation.

**P10.02****Application of Next Generation Sequencing (NGS) in the ALS research: a study in Swedish families**R. Rofougaran<sup>1</sup>, L. Köhn<sup>2</sup>, A. Birve<sup>1</sup>, A. Nilsson<sup>1</sup>, M. Berdyski<sup>1</sup>, P. Andersen<sup>1</sup>,<sup>1</sup>Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden, Umeå, Sweden, <sup>2</sup>Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden, Umeå, Sweden.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that eventually leads to the death of motor neurons and fatal paralysis. Approximately, mutations in 26 genes have been associated with pathogenesis of ALS and several of them are also linked with fronto-temporal dementia (FTD). However, mutations in these genes explain less than one-third of ALS cases. NGS technologies can be used to generate deep sequencing of target genome regions, such as the exome or known disease loci. Generally, the specificity and accuracy of the results from panels are more reliable compared to whole-exome sequencing data since panels have better and more efficient data coverage than whole-exome sequencing. The aim of the current project is to analyze 26 genes that are associated with ALS disease using NGS panel. After library and indexed-enriched library preparations and validations of samples who had previously tested negative for C9orf72 mutations, the samples were sequenced on MiSeq. Analyzing the data showed the sufficient sequencing with mean coverage of more than 150X. Then, bioinformatics analyzes were done and the coding and splicing variants were selected and filtered against MAF below 1% which have been described in HGMD. Our results showed mutations in SOD1 and other ALS-linked genes as well. The results will be discussed in more detail. To conclude, the combination of DNA capture enrichment systems and high-throughput sequencing techniques can be used to have deep insight for the diagnosis of ALS.

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**P10.03****Study of the role of epigenetic regulators in the pathogenesis of ALS**

C. Bernardini, M. Bernardinelli, M. Branzini, L. Di Pietro, F. Michetti, E. Ricci, M. Sabatelli;

Università Cattolica del Sacro Cuore, Rome, Italy.

ALS is characterized by the progressive loss of neuromuscular junction (NMJ) and muscle mass, followed by muscle weakness, paralysis and death. Most of the studies on ALS focused primarily on motor neurons (MN) to understand the etiology of the disease. However, there are evidences suggesting that muscle dysfunction and NMJ degeneration occur long before symptom onset and may contribute to MN death. Recently, HDAC4 was identified as the critical linker connecting neural activity to muscle transcription; indeed induction of HDAC4 leads to increased levels of myogenin. Moreover the regulation of RNA metabolism in the NMJ is an emerging mechanism involved in ALS pathogenesis, and microRNAs (miRNAs) could be the mediators of this process. The aim of this study is to unravel the molecular pathway that exists between nerve and muscle in order to control the mechanisms that exacerbate the disease. In particular selected genes and miRNAs involved in the innervation pathway have been analyzed in human skeletal muscle derived from ALS patients. 12 ALS patients with 7 relative controls have been collected for this aim. Selected genes and miRNAs were then analyzed through qPCR and differential expression of HDAC4, MYOG, mir-23a, mir-155 and mir-206 was found. Collectively these data suggest that the innervation pathway is modulated in skeletal muscle in ALS patients and identify specific miRNAs potentially acting in HDAC4 inhibition. The inhibition of HDAC4 could represent a promising therapeutic approach to enhance motor performance and slow disease progression in patients with ALS.

This study has been funded by Arisla

**P10.04****Confirming FIG4 as a risk gene for amyotrophic lateral sclerosis (ALS) in a central European cohort by whole exome and targeted sequencing**I. Rangnau<sup>1,2</sup>, A. Osmanovic<sup>1,2</sup>, A. Kosfeld<sup>1</sup>, S. Abdulla<sup>2</sup>, C. Janssen<sup>2</sup>, S. Petri<sup>2</sup>, R. G. Weber<sup>1</sup>,<sup>1</sup>Department of Human Genetics, Hannover Medical School, Hannover, Germany,<sup>2</sup>Department of Neurology, Hannover Medical School, Hannover, Germany.

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease characterized by progressive degeneration of upper and lower motor neurons. It leads to increasing weakness of voluntary muscles until death occurs from respiratory failure within an average of three years. While important progress has been made in understanding the genetic etiology of ALS, the majority of genetic variation involved in ALS is still undefined. In this study, we performed whole exome sequencing (WES) in an ALS

family showing an autosomal dominant inheritance pattern with incomplete penetrance, comprising an index patient and his affected paternal great-aunt. By using an overlapping strategy to identify variants shared by the patient and his father, we detected a rare heterozygous frameshift mutation in *FIG4* predicted to truncate the FIG4 protein in its active site. *FIG4* encodes a phosphoinositide 5-phosphatase with a key role in vesicle trafficking in eukaryotic cells. Bi-allelic *FIG4* mutations have previously been described to cause Charcot-Marie-Tooth disease, type 4J (CMT4J) and Yunis-Varon syndrome. Recent studies showed an association between heterozygous *FIG4* mutations and ALS. Therefore, we performed WES or targeted sequencing of *FIG4* in 200 ALS patients of mainly central European origin revealing five additional known or novel rare heterozygous missense mutations predicted to be deleterious. Disease duration was relatively long in four of six *FIG4* mutation carriers, and three of six displayed a predominant upper motor neuron phenotype. Here, we confirm *FIG4* as an ALS risk gene identifying novel or known rare heterozygous variants in 3% of European patients.

**P10.05****A syndromic form of Charcot-Marie-Tooth disease associated with a 41-Mb terminal duplication on Xp22.3-11.4 spanning the PDX3 gene (CMTX6)**A. Pelle<sup>1,2</sup>, D. Carli<sup>1,2</sup>, E. Di Gregorio<sup>3,4</sup>, G. Mandrile<sup>1</sup>, B. Ferrero<sup>5</sup>, M. De Marchi<sup>1,2</sup>, D. F. Giachino<sup>1,2</sup>, A. Brusco<sup>3,4</sup>,<sup>1</sup>University of Torino, Department of Clinical and Biological Sciences, Torino, Italy,<sup>2</sup>Orbassano (TO), Italy, <sup>3</sup>Medical Genetics, San Luigi Gonzaga University Hospital,<sup>4</sup>S.C.D.U. Medical Genetics, Città della Salute e della Scienza, Torino, Italy, <sup>5</sup>University of Torino, Department of Medical Sciences, Torino, Italy,<sup>6</sup>University Division of Neurology, San Luigi Gonzaga University Hospital, Orbassano (TO), Italy.

We report on a mother and her daughter affected by peripheral polyneuropathy and intellectual disability. The two patients referred frequent falls and walking difficulties. A motor- mixed sensory axonal-demyelinating polyneuropathy was diagnosed with electromyography (MCV: 33m/sec in the mother, 25m/sec in the daughter). Clinical features included in both patients premature ovarian failure, osteoporosis, body freckling, pes cavus and claw toes. Early hair whitening, seizures and thyroid nodular hyperplasia were observed only in the daughter.

In both subjects, a telomeric duplication at Xp22.3-11.4 was identified by array-CGH analysis. The duplicated region spanned 41 Mb and encompassed 135 genes, among which only few had previously been associated with some aspects of their clinical phenotype.

Duplications of CDKL5 and RPS6KA3 cause developmental delay and learning disabilities both in males and females. SAT1 duplication is associated with a rare kind of chronic dermatitis named keratosis follicularis spinulosa decalvans. Interestingly, PDK3 had been associated with the new X-linked dominant Charcot-Marie-Tooth type 6 (CMTX6, MIM300905) on the basis of the finding of a missense PDK3 (p.Arg158His) gain-of-function mutation (Kennerson et al, 2013). Thus, in our patients we hypothesize an activating role of the PDK3 duplication, in analogy with PMP22 in the Charcot-Marie-Tooth type 1A.

In conclusion, we describe two patients with a 41-Mb Xp-telomeric duplication related to a syndromic form of polyneuropathy and intellectual disability. Clinical phenotype could be explained by a combination of duplicated genes: CDKL5 and RPS6KA3 for intellectual disability, SAT1 for body freckling, PDK3 for polyneuropathy.

**P10.06****A novel mutation in NEFL gene associated with early onset severe Charcot-Marie-Tooth disease in Karachay populations**R. A. Zinchenko<sup>1,2</sup>, E. L. Dadali<sup>1,2</sup>, A. K. Makaov<sup>3</sup>, A. V. Polyakov<sup>1</sup>, M. V. Bulach<sup>1</sup>, E. K. Ginter<sup>1</sup>,<sup>1</sup>Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>2</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation, <sup>3</sup>Municipal Budgetary Health Care setting, Chabuz, Russian Federation.

Introduction: Charcot-Marie-Tooth (CMT) - is genetically and clinically heterogeneous group of the peripheral nervous system diseases. On average, in the world their prevalence is 10.0 per 100,000 persons, ranging from 0.1 to 41.0 per 100,000 persons.

Materials and Methods: During expeditions into Ust-Dzhegutinsky district of Karachai-Cherkess 10 patients from one family with autosomal dominant form CMT were identified. We developed and used the panel for TargetSeq containing 400 genes mutations in which are found by NGS of neurogenetic diseases patients. The analysis was performed on Illumina NextSeq 500 by paired-end reads (2x151 bp) with an average coverage of at least 70-100x

paired-end.

Results: Using NGS scanning, we identified heterozygous substitution c.65C>A (p.Pro22His) in exon 1 of the gene NEFL. This nucleotide substitution has not been previously reported in literature and mutations databases (HGMD, IPNMDB). Then we searched the mutation in other patients, using the method of Sanger sequencing. The mutation c.65C>A was detected in all affected family members in the heterozygous state. The debut of the disease between 13 to 18 years with the appearance of weakness peroneal muscle groups. The speed of the pulse (SPI) on the median nerve in patients was from 33.5 to 42.3 m/s.

Conclusion: This is the first report of missense mutations c.65C>A (p.Pro22His) gene NEFL, associated with a group of intermediate types CMT. This types CMT was found in Karachai family. The prevalence in the region amounts 1:3300 Karachai.

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#### P10.08

##### Prevalence and spectrum of SH3TC2 mutations in Norway

K. A. Arntzen<sup>1</sup>, H. Høyér<sup>2</sup>, M. Ingebrigtsen<sup>3</sup>, Ø. L. Busk<sup>2</sup>, Ø. L. Holla<sup>2</sup>, K. Tveten<sup>2</sup>, H. T. Hilmarsen<sup>2</sup>, K. Ørstavik<sup>4</sup>, C. Tallaksen<sup>4,5</sup>, M. Nebuchennykh<sup>1</sup>, C. Jonsrud<sup>3</sup>, G. J. Braathen<sup>2</sup>, T. Fagerheim<sup>3</sup>;

<sup>1</sup>Department of Neurology, University Hospital of North Norway, Tromsø, Norway,

<sup>2</sup>Section of Medical Genetics, Telemark Hospital, Skien, Norway, <sup>3</sup>Department of Medical Genetics, University Hospital of North Norway, Tromsø, Norway, <sup>4</sup>Department of Neurology, Oslo University Hospital, Oslo, Norway, <sup>5</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway.

Charcot-Marie-Tooth disease type 4C (CMT4C) is a demyelinating recessive form of CMT, caused by mutations in the SH3TC2 gene. CMT4C has so far been regarded as a relatively uncommon type of CMT and the literature describes mostly single cases and families. The aim of this study was to investigate the prevalence and spectrum of SH3TC2 mutations in the Norwegian CMT population, and to characterize the clinical manifestations of this patient group.

This project is a co-operation between the two centers in Norway diagnosing CMT4C. All patients diagnosed with homozygous or compound heterozygous mutations in the SH3TC2 gene were invited to participate in the study. The study is based on genetic analysis, information from clinical health records and patient questionnaires.

In total 35 patients from 30 families were diagnosed with homozygous or compound heterozygous mutations in SH3TC2. Most common was the NM\_024577.3: c.2860C>T p.(Arg954\*) mutation, present in 21 patients as homozygous, and in 13 patients as heterozygous in combination with another SH3TC2 mutations. The additional mutations included among others seven mutations not previously reported in the literature. To date 20 patients have been included in the clinical study. Mean age of onset was four years, affection of arms, scoliosis and reduced hearing were reported by 95, 80 and 50% of the patients respectively.

Mutations in SH3TC2 are one of the five most frequent causes of CMT in the Norwegian CMT population. All patients except one, carried one or two c.2860C>T mutations, a variant which likely has high carrier frequency in Norway.

#### P10.09

##### DM2-linked myopathy caused by uninterrupted short (CCTG)50-70 repeat expansion in CNBP

T. Suominen<sup>1</sup>, L. Bachinski<sup>2</sup>, O. Raheem<sup>1</sup>, H. Haapasalo<sup>3</sup>, W. Kress<sup>4</sup>, R. Krahe<sup>2,5</sup>, B. Udd<sup>1,6</sup>;

<sup>1</sup>University of Tampere, Neuromuscular Research Unit, Tampere, Finland, <sup>2</sup>Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX, United States, <sup>3</sup>Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Tampere, Finland, <sup>4</sup>Institute of Human Genetics, University of Wuerzburg, Biozentrum, Am Hubland, Wuerzburg, Germany, <sup>5</sup>Graduate Programs in Human and Molecular Genetics and Genes and Development, University of Texas at Houston Graduate School of Biomedical Sciences, Houston, TX, United States, <sup>6</sup>Department of Neurology, Tampere University Hospital, Tampere, Finland.

Background and aims: Myotonic dystrophy type 2 (DM2) is one of the most common adult-onset muscular dystrophies in Europe. It is a dominantly inherited multisystemic disease caused by a repeat expansion in intron 1 of CNBP gene. The disease-causing repeat expansions consist of 75-11,000 (CCTG) repeats. In this study, we characterize a new type of DM2-linked mild myopathy in several patients without myotonia associated with very short (CCTG)<sub>50-70</sub> repeat expansions.

Methods: All ten individuals carrying a short (CCTG) repeat expansion have been analyzed by repeat-primed PCR and haplotype analysis. Additionally five patients have been clinically evaluated and muscle biopsy analyzed. For one of the patient, an index patient of a larger family, the mutation has been

studied further by Southern blot, sequencing, allele specific expression, *in situ* hybridization, Western blot and analysis of fetal splice isoforms of DM2 effector proteins.

Results: Clinical features are mild, including myalgic pain, muscle weakness and stiffness. Muscle biopsy showed DM2-like changes. PCR-based methods and Southern blot indicated a repeat expansion of (CCTG)<sub>50-70</sub>. In contrast to DM2, ribonuclear foci were not detected, splicing of DM2 effector proteins was normal and CNBP protein level was normal.

Discussion and conclusions: Finding of (CCTG)<sub>50-70</sub> repeats containing mutations in mildly symptomatic patients suggests that even shorter than previously reported expansions may cause a disease, although with a different phenotype. The molecular findings indicate a unique pathomechanism and the term 'myotonic dystrophy' cannot be applied to this new type of disease.

#### P10.10

##### Multi-gene panel analysis in the primary diagnosis of limb-girdle muscular dystrophy

V. Mayer<sup>1</sup>, S. Bulst<sup>1</sup>, A. Nissen<sup>1</sup>, S. Kleinle<sup>1</sup>, K. Becker<sup>1</sup>, M. C. Walter<sup>2</sup>, A. Benet-Pagès<sup>1</sup>, A. Abicht<sup>1,2</sup>;

<sup>1</sup>Medizinisch Genetisches Zentrum MGZ, Munich, Germany, <sup>2</sup>Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Germany.

Introduction: Limb-girdle muscular dystrophies are characterized by great clinical and genetic heterogeneity. Mutations in a number of genes lead to overlapping phenotypes that cannot always be differentiated clinically or through muscle biopsy. Molecular genetic testing is therefore becoming increasingly important to the initial diagnosis of potential dystrophinopathies with significant clinical and familial consequences.

Materials and Methods: Our cohort comprised more than 80 patients with a suspected differential diagnosis of dystrophinopathy and negative test results for DMD gene dosage analysis by MLPA and DMD gene point mutation analysis by next generation sequencing (NGS). As over 700 neurogenetic/neuromuscular genes were captured during the course of technical analysis by NGS, we were able to perform a second-tier expanded data analysis of a gene panel including more than 140 genes known to be responsible for congenital muscular dystrophies or myopathies.

Results: More than 30% of patients tested were found to have a disease-causing mutation in another gene. In other patients sequence variants of unknown clinical significance were detected; the potential clinical relevance of these variants cannot be assessed with certainty without further testing.

Conclusion: Multi-gene panels utilizing NGS technologies enable sensitive, cost-efficient, and simultaneous analysis of multiple disease-relevant genes. However, despite the broad analysis, it is not possible to identify pathogenic mutations in all patients. There is also a risk that the analysis of a large number of genes independent of the clinical phenotype may identify variants that can be assessed only in combination with refined clinical or muscle biopsy data and/or segregation analysis.

#### P10.11

##### Deletion and duplication patterns of dystrophin gene in Turkish Duchenne/Becker muscular dystrophy patients

F. Guclu-Geyik<sup>1</sup>, N. Coban<sup>1</sup>, B. Tüysüz<sup>2</sup>, P. Serdaroglu<sup>3</sup>, Y. Parman<sup>4</sup>, F. Deymeer<sup>4</sup>, N. Erginol-Unaltuna<sup>1</sup>;

<sup>1</sup>Istanbul University, Institute for Experimental Medicine, Department of Genetics, ISTANBUL, Turkey, <sup>2</sup>Istanbul University Cerrahpaşa Faculty of Medicine, Department of Pediatric Genetics, ISTANBUL, Turkey, <sup>3</sup>Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, ISTANBUL, Turkey, <sup>4</sup>Istanbul University, Istanbul Medical Faculty, Department of Neurology, ISTANBUL, Turkey.

Background: Duchenne and Becker muscular dystrophies (DMD/BMD) are X-linked recessive inherited neuromuscular diseases that result from heterogeneous mutations in the dystrophin (DMD) gene. Our aim was to investigate DMD gene deletion/duplication frequencies and patterns in Turkish DMD/BMD patients and carriers.

Methods: Our study comprised of 217 male patients clinically diagnosed or suspected with DMD/BMD and 125 female cases. DMD gene deletion/duplications were detected by Multiplex ligation-dependent probe amplification (MLPA) method.

Results: DMD gene mutations were identified in 44.7% of male probands, of which 36.4% (mean age of diagnosis: 9.48, range; from 0 to 32 age) and 8.3% (mean age of diagnosis 11.78, range; from 2 to 35 age) were deletions and duplications, respectively.

There were 45 deletions, 11 of which were single exon deletions. Duplication pattern however, were found in 17 male patient. Deletions of exons 45-47 (n=6) and 45-50 (n=5) were the most common identified patterns. The range of the duplications in our group was mainly between exons 2-27 and single exon duplications were mostly located in the central region (51-53)

of DMD gene. In the females however, the carrier status was determined to be 27.2%. Interestingly, the DMD mutation was inherited from the mother in 63%, 37% were sporadic cases.

**Conclusion:** This is the first well-established deletion/duplication database in Turkey. MLPA is a sensitive and useful technology which allows detection of DMD deletions/duplications in affected males and carrier mothers, also offers appropriate genetic counseling in Turkey.

## P10.13

### MLPA analysis and clinical profile of DMD patients in Turkish population

M. Y. Alp<sup>1</sup>, A. Yesilyurt<sup>1</sup>, F. A. Pinarli<sup>1</sup>, G. Haliloglu<sup>2</sup>, B. Konuskan<sup>2</sup>, H. Topaloglu<sup>2</sup>;

<sup>1</sup>Diskapi Yildirim Beyazit Research and Training Hospital Genetic Diagnostic Center, Ankara, Turkey, <sup>2</sup>Hacettepe University, Faculty of Medicine, Division of Pediatric Neurology, Ankara, Turkey.

Duchenne muscular dystrophy (DMD) is the most common muscular disease in children, and one of the most frequent genetic disorder. It has an incidence of 1:3,500 live born males with a prevalence of 6 in 100000 males. DMD is inherited in X-linked recessive manner caused by mutations in dystrophin gene (DMD) which is the largest human gene encoding dystrophin protein. DMD gene located at Xp21, contains 79 exons, spanning 2.4 Mb of a genomic sequence. New therapeutic strategies e.g. exon skipping made genetic diagnosis crucial for patients. In Genetic Diagnostic Center of Diskapi Yildirim Beyazit Training and Research Hospital, 238 clinically diagnosed male DMD patients were examined by MLPA for detecting deletion or duplication in DMD gene. 154 patients (64.7%) had abnormal MLPA results, 140 deletion (58.8%), 14 duplication (5.9%), 84 patients (35.3%) were normal. Most affected exons were exon 50, 46, and 47, respectively. 66.2 percent of MLPA mutations were disrupted readingframe which were candidate of exon skipping. DMD patients were also characterized as follows; mean current age was 8.8 years, mean age at diagnosis was 4.7 years, mean CK levels at diagnosis was 11193 U/L. At diagnosis, 30.5% of patients had asymptomatic CK elevation, 69.5% of patients were symptomatic. 11.8 percent of patients had wheelchair dependency. 29.6 percent of patients had family history, 57 percent of patients had carrier mother. Statistical analysis revealed that positive family history was significantly associated with normal MLPA results, and wheelchair dependency was significantly associated with presence of exonic deletion and duplication.

## P10.14

### Molecular diagnosis of Duchenne muscular dystrophy: Best practices and guidelines for molecular testing and test results reporting with advent of molecular therapies

A. Ferlini<sup>1</sup>, A. Aartsma-Rus<sup>2</sup>, M. Bailey<sup>3</sup>, P. Bayrak-Toydemir<sup>4</sup>, Y. Chien<sup>5</sup>, M. Davis<sup>6</sup>, M. Foncuberta<sup>7</sup>, F. Giliberto<sup>8</sup>, I. Ginjaar<sup>9</sup>, L. Hagerty<sup>10</sup>, M. Hegde<sup>11</sup>, E. Izzo<sup>12</sup>, E. Kimura<sup>12</sup>, A. Kiselev<sup>13</sup>, L. Kulikowski<sup>14</sup>, J. Kwon<sup>15</sup>, J. Lee<sup>16</sup>, A. Lindy<sup>17</sup>, A. Martin<sup>18</sup>, S. Monges<sup>7</sup>, C. Müller<sup>19</sup>, J. Nectoux<sup>20</sup>, R. Santos<sup>21</sup>, T. Sejersen<sup>22</sup>, J. Scull<sup>23</sup>, H. van Duyvenvoorde<sup>9</sup>, F. Varfaj<sup>3</sup>, E. Welch<sup>24</sup>, S. Yau<sup>25</sup>, E. Zanotelli<sup>26</sup>, N. Miller<sup>3</sup>;

<sup>1</sup>Department of Medical Sciences, Medical Genetics Unit, Università degli Studi di Ferrara, Ferrara, Italy, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands,

<sup>3</sup>BioMarin Pharmaceutical, Inc., Novato, CA, United States, <sup>4</sup>University of Utah School of Medicine, ARUP Laboratories, Salt Lake City, UT, United States, <sup>5</sup>Department of Medical Genetics and Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, <sup>6</sup>PathWest Laboratory Medicine WA, Perth, Australia, <sup>7</sup>Hospital Pediátria J.P. Garrahan, Buenos Aires, Argentina, <sup>8</sup>Hospital de Clínicas José San Martín, Buenos Aires, Argentina, <sup>9</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>10</sup>Muscular Dystrophy Association, Durham, NC, United States,

<sup>11</sup>Emory Genetics Laboratory, Decatur, GA, United States, <sup>12</sup>Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan, <sup>13</sup>D.O.Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russian Federation,

<sup>14</sup>Department of Pathology, University of São Paulo, São Paulo, Brazil, <sup>15</sup>Departments of Neurology, Golisano Children's Hospital at Strong Memorial Hospital, University of Rochester Medical Center, Rochester, NY, United States, <sup>16</sup>Greenwood Genetic Center, Greenwood, SC, United States, <sup>17</sup>Neurogenetics Department, GeneDx, Gaithersburg, MD, United States, <sup>18</sup>Duchenne Connect, Parent Project Muscular Dystrophy, Hackensack, NJ, United States, <sup>19</sup>Department of Human Genetics, University of Würzburg, Würzburg, Germany, <sup>20</sup>Laboratoire de Biochimie et Génétique Moléculaire, Hospital Cochin, Paris, France, <sup>21</sup>Department of Genetics, Centro de Genética Médica Jacinto Magalhães, CHP, Porto, Portugal, <sup>22</sup>Department of Women's and Children's Health, Stockholm, Sweden,

<sup>23</sup>Medical Genetics Laboratory, Baylor College of Medicine, Houston, TX, United States, <sup>24</sup>PTC Therapeutics, South Plainfield, NJ, United States, <sup>25</sup>Department of Genetics, Genetics Viapath LL

**Background/Objectives:** Duchenne muscular dystrophy (Duchenne/DMD) is a severe form of muscular dystrophy caused by mutations in the DMD gene. Molecular testing is essential for an early and accurate diagnosis. Two working groups of international molecular testing experts, organized by BioMarin, met to evaluate the current state of Duchenne/DMD diagnostic

molecular testing and results reporting.

**Methods:** Twenty-seven Duchenne/DMD experts, including molecular/clinical geneticists, research scientists, and genetic counselors, met in July and November, 2015 to discuss Duchenne/DMD molecular diagnosis and reporting. Representatives from 20 diagnostic laboratories also completed a comprehensive survey.

**Results:** Laboratories detect large deletions and duplications (~80% of pathogenic DMD mutations) by one or more methods: multiplex ligation-dependent probe amplification (MLPA, 90% of laboratories surveyed), array comparative genomic hybridization (aCGH, 40%), and next generation sequencing (NGS, 50%). Few laboratories still use multiplex PCR (mPCR). All these methods, except mPCR, are sufficient to detect deletions/duplications in DMD; sequence-level mutation detection requires use of Sanger sequencing or NGS. For molecular therapy-eligible Duchenne/DMD, precise molecular analysis, accurate result interpretation, and clear reporting are essential to inform healthcare professionals.

**Discussion:** The experts recommend an updated molecular diagnostic algorithm with tiered testing options to guide the accurate detection of all DMD mutations and determination of molecular therapy eligibility. MLPA-based single exon deletion/duplication results must be confirmed by an alternative method. If a deletion/duplication is not detected, sequencing is recommended. The experts recommend revising best practices for DMD molecular testing, interpretation, and results reporting due to new developments in molecular diagnostics and therapies in development.

## P10.16

### Genetic and clinical heterogeneity of dystroglycanopathies: challenging for next-generation sequencing?

J. Oliveira<sup>1,2</sup>, S. Janssens<sup>3</sup>, A. Gonçalves<sup>1,2</sup>, E. Vieira<sup>1,2</sup>, M. E. Oliveira<sup>1,2</sup>, T. Moreno<sup>4</sup>, L. Negrão<sup>5</sup>, I. Fineza<sup>6</sup>, M. Sousa<sup>7,8</sup>, R. Santos<sup>1,2,9</sup>,

<sup>1</sup>Unidade de Genética Molecular, Centro de Genética Médica Dr Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal, <sup>2</sup>Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Porto, Portugal, <sup>3</sup>Centro for Medical Genetics, Ghent University Hospital, Ghent, Belgium,

<sup>4</sup>Unidade de Neuropediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisboa, Portugal, <sup>5</sup>Consulta de Doenças Neuromusculares, Hospitais da Universidade de Coimbra, Centro Hospitalar Universitário de Coimbra, Centro Hospitalar do Porto, Coimbra, Portugal, <sup>6</sup>Unidade de Neuropediatria, Centro de Desenvolvimento da Criança Luís Borges, Hospital Pediátrico de Coimbra, Centro Hospitalar Universitário de Coimbra, Coimbra, Portugal, <sup>7</sup>Departamento de Microscopia, Laboratório de Biologia Celular, Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal, <sup>8</sup>Centro de Genética da Reprodução Prof. Alberto Barros, Porto, Portugal, <sup>9</sup>UCIBIO/REQUIMTE, Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

**Introduction:** Dystroglycanopathies, a group of diseases defined by hypoglycosylation of alpha-dystroglycan, are classified into three major subtypes: severe congenital muscular dystrophy (MD) with brain and eye anomalies (type A), congenital MD with mental retardation (type B) and limb-girdle MD (type C). Eighteen different genes have been linked to dystroglycanopathies, the majority coding for proteins involved in O-mannosylglycan biosynthesis. Recently, defects in the synthesis of dolichol phosphate mannose, initially associated with congenital disorders of glycosylation, was shown to cause dystroglycanopathy.

**Materials and Methods:** Twenty-one patients with suspected dystroglycanopathy – ranging from severe congenital forms to milder adult-onset MD – were genetically characterized by Sanger sequencing, using a stepwise approach or homozygosity mapping. MLPA and/or cDNA analysis was performed in four cases.

**Results:** Eleven distinct pathogenic variants were identified in four genes: *FKRP* (5 missense and one base-pair duplication, 16 patients), *POMGNT1* (2 variants affecting splicing and 1 missense, 3 patients), *POMT2* (1 missense variant, 1 patient) and *FKTN* (1 multi-exonic duplication, 1 patient). These comprise four unpublished variants: two missense (p.Tyr407Asp and p.Asp360His both in *FKRP*), one frameshift (*FKRP*:c.1234dupC) and one apparently silent substitution causing exon skipping (*POMGNT1*:c.534G>A/r.421\_534del).

**Conclusions:** The expanding clinical and genetic heterogeneity of dystroglycanopathies could justify the application of comprehensive next-generation sequencing (NGS) applications such as whole-exome sequencing. Interestingly, we found four patients with pathogenic alleles that could be problematic for NGS automated analysis pipeline. Thus, it is essential to employ robust analytical algorithms for data analysis and approaches focusing on RNA to successfully characterize these patients.

**P10.17****Multifactor dimensionality reduction: detection gene-gene interactions in DMD studies**

V. C. Sacara;

Institute of Mother and Child, Chisinau, Moldova, Republic of.

Epistasis is increasingly assumed to play a crucial role in the architecture of genetics disease. We try to modeling epistasis between 5 genes: dystrophin, MTHFR, MTR, MTRR, eNOS in case of the wheelchair dependency- up to 9or12 years.

A retrospective long-term study was carried out in 148 corticosteroids-free DMD patients. Gene-gene interactions were analyzed using entropy-based MDR 3.02.

The analysis of the hierarchical structure of all components, the best model in case of 9wch consists of four interconnected clusters. The first cluster is presented independent of the dystrophin deletion from other genes. The second cluster also integrates with MTRR gene locus independently interconnected clusters combine the two genes clusters: cycle folate (MTHFR677,MTHFR1298) and MTR-eNOS (both strong synergy), having the effect of interaction between each other- moderate synergy. In group of 12wch, we have identified two separate clusters. The first independent cluster has united the groups of related loci MTHFR1298 and eNOS expressed duplicate the effects of interactions. The second cluster has established a strong synergy between MTHFR677 locus, the dystrophin deletion and MTR, in which the activity of dystrophin is depended from MTR. In the circular graph was found that the effect of MTHFR677 at 12wch decreasing (0.16%), compared with the effect in 9wch(1.78%), also noted the impact of the MTR (0.48) diminishes as compared with the effect of this locus at 9wch(1.08%). But in the 12wch has increased the impact of MTHFR1298 locus(1.96%) and information gain of pair «MTHFR1298-eNOS»-1.64%. This method can extend the biological reach of pathway-based results

**P10.18****Intergenerational instability in Huntington's disease: insights from mice models**J. L. Neto<sup>1,2</sup>, J. Lee<sup>1</sup>, T. Gillis<sup>1</sup>, J. R. Guide<sup>1</sup>, B. Lager<sup>3</sup>, I. Alonso<sup>2,4</sup>, V. C. Wheeler<sup>1</sup>, R. Mouro-Pinto<sup>1</sup>;

<sup>1</sup>Center for Human Genetic Research - Massachusetts General Hospital, Boston, MA, United States, <sup>2</sup>UnIGENE, IBMC – Instituto de Biologia Molecular e Celular and i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal, <sup>3</sup>CHDI Foundation Inc, Princeton, NJ, United States, <sup>4</sup>Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal.

Intergenerational CAG repeat instability is a distinctive feature of Huntington's disease (HD), associated with genetic anticipation. Intergenerational repeat changes are present in patients and mouse models of the disorder. In this work two large breeding datasets of HD knock-in mouse models, globally encompassing more than 18,000 repeat transmissions, led us to confirm and gain new insight into factors influencing intergenerational instability.

The first dataset includes information from 16,000 transmissions across five lines ( $Htt^{080}$ ,  $Htt^{092}$ ,  $Htt^{0111}$ ,  $Htt^{0140}$ ,  $Htt^{0175}$ ) and allowed us to determine effects of 1) parent-of-origin; 2) sex of offspring and 3) parental CAG repeat length on the frequency and magnitude of unstable transmissions. Parent of origin, but not the sex of the offspring contributed to intergenerational instability. We also determined that paternal CAG size affects the frequency of expansions and stable transmissions, while not significantly altering the rate of contractions, even though the magnitude of both contractions and expansions are affected by CAG length. Importantly, the sizeable dataset revealed the presence of large repeat length changes as seen in patients and not previously appreciated in mouse models.

The second dataset is comprised of about 2,000 transmissions from knock-in mice belonging to six different background strains (129, CD1, FVB, DBA, B6N, B6J), providing insight into effects of genetic background on the frequency of unstable transmissions independent from parental CAG size effects. These results provide novel opportunities to search for CAG instability modifiers through the comparison of genetic variation between different strains.

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**P10.19****A new early-onset myopathy associated with deficiency in kyphoscoliosis peptidase (KY)**C. Hedberg-Oldfors<sup>1</sup>, N. Darin<sup>1</sup>, M. Olsson Engman<sup>2</sup>, A. Oldfors<sup>1</sup>;

<sup>1</sup>University of Gothenburg, Gothenburg, Sweden, <sup>2</sup>Blekinge Hospital, Karlskrona, Sweden.

We describe a new congenital myopathy due to a homozygous loss-of-func-

tion mutation in the kyphoscoliosis peptidase gene (KY). We investigated a 7.5-year-old girl with walking difficulties from two years of age presented with generalized muscle weakness; mild contractures in the shoulders, hips, and feet; cavus feet; and lordosis but no scoliosis. She had previously been operated with Achilles tendon elongation. Whole-body MRI showed atrophy and fatty infiltration in the calf muscles. Biopsy of the vastus lateralis muscle showed variability in fiber size, with some internalized nuclei and numerous very small fibers with variable expression of developmental myosin heavy chain isoforms. Some small fibers showed abnormal sarcomeres with thickened Z-discs and abortive nemaline rods. Whole-exome sequencing revealed a homozygous one-base deletion (c.1071delG, p.T358fs\*3) in KY, predicted to result in a truncated protein. Analysis of an RNA panel revealed that KY is predominantly expressed in skeletal muscle in humans. A recessive mutation in the murine ortholog *Ky* was previously described in a spontaneously generated mouse mutant with kyphoscoliosis, which developed postnatally and was caused by dystrophy of postural muscles. We describe the first human case of disease associated with KY inactivation. As in the mouse model, the affected child showed myopathy and muscle weakness—but in contrast, no kyphoscoliosis.

**P10.20****AN05 mutations identified in the Polish LGMD patients by whole exome sequencing**J. P. Fichna<sup>1</sup>, A. Macias<sup>2</sup>, M. Suszek<sup>3</sup>, A. Maruszak<sup>1</sup>, M. J. Rędowicz<sup>2</sup>, A. M. Kamińska<sup>2</sup>, C. Żekanowski<sup>1</sup>;

<sup>1</sup>Department of Neurodegenerative Disorders, Mossakowski Medical Research Centre, Warsaw, Poland, <sup>2</sup>Department of Neurology, Medical University of Warsaw, Warsaw, Poland, <sup>3</sup>Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland.

The anoctamin-5 belongs to a family of proteins involved in a variety of cellular functions including chloride ion transport. The precise function of the anoctamin-5 protein remains largely unknown, however expression pattern suggests its important role in the musculoskeletal system. Recessive *AN05* gene mutations cause limb-girdle muscular dystrophy (LGMD) and Miyoshi myopathy (MMD3). Here, we describe *AN05* mutations found in LGMD patients in Poland.

The study group comprised probands representing 82 families with a clinical diagnosis of LGMD. To find causative mutations and comprehensively analyse genetic background of the disease we have determined exomic sequence using Whole Exome Sequencing (WES) method.

We have identified eight different *AN05* mutations, two of them novel, in seven patients. Two patients presented compound heterozygosity and carried mutations that could be considered pathogenic. In three cases known single known *AN05* mutations accompany pathogenic variants in *DYSF* and *CAV3*, and might only have phenotype modifying effect. In one case another single, although novel and putatively pathogenic *AN05* mutation, was identified and further investigation of genetic etiology is needed.

Our study reveals that *AN05* mutations in the Polish population have lower prevalence compared to the Northern Europe, where it is the second most common cause of LGMD. Additional mutations found apart from the most likely causative ones might also influence the phenotype. WES can be applied as a single genetic test to quickly characterize the comprehensive spectrum of genetic variation.

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**P10.21****A novel mutation in the desmin gene (DES) cause an autosomal recessive form of limb-girdle muscular dystrophy type 2R without clear-cut desminopathy pathology**B. Balci-Hayta<sup>1</sup>, N. Cetin<sup>1</sup>, H. Gundesli<sup>1</sup>, P. Korkusuz<sup>2</sup>, N. Purali<sup>3</sup>, B. Talim<sup>4</sup>, E. Tan<sup>5</sup>, D. Selcen<sup>6</sup>, S. Erdem-Ozdamar<sup>5</sup>, P. Dincer<sup>4</sup>;

<sup>1</sup>Hacettepe University Faculty of Medicine, Department of Medical Biology, Ankara, Turkey, <sup>2</sup>Hacettepe University Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey, <sup>3</sup>Hacettepe University Faculty of Medicine, Department of Biophysics, Ankara, Turkey, <sup>4</sup>Hacettepe University Faculty of Medicine, Department of Pediatrics, Pathology Unit, Ankara, Turkey, <sup>5</sup>Hacettepe University Faculty of Medicine, Department of Neurology and Neuromuscular Diseases Research Laboratory, Ankara, Turkey, <sup>6</sup>Mayo Clinic, Department of Neurology and Neuromuscular Disease Research Laboratory, Rochester, NY, United States.

**Introduction:** The autosomal recessive forms of limb girdle muscular dystrophies (LGMD2) are a group of rare genetic disorders that are characterized by progressive muscle weakness of the shoulder and pelvic girdle muscles and include at least 23 different genetic entities.

**Materials and Results:** Genome-wide homozygosity mapping was perfor-

med in a consanguineous LGMD2 family with two affected individuals and the LGMD2 phenotype was mapped to chromosome 2q35-q36.3. DNA sequence analysis of the candidate gene DES revealed a homozygous splice site mutation c.1289-2A>G in the affected family members. IF staining and WB analysis showed that the expression and the cytoskeletal network formation of mutant desmin were preserved in skeletal muscle fibres. Interestingly, the affected individuals do not have the classical features of desminopathy, neither cardiomyopathy nor the typical histological changes such as disruption of myofibrillar organization, aggregation of intracellular proteins, dislocation/aggregation of membranous organelles. This novel mutation results in addition of 16 amino acids within the tail domain of desmin, which has been suggested to interact with lamin B protein. We also detected a specific disruption of desmin-lamin B interaction in the skeletal muscle of the patient by confocal laser scanning microscopy.

Conclusions: DES mutations should be considered as a cause of LGMD2 without features of myofibrillar myopathy and the inability of mutant desmin to interact with lamin B may trigger disease development. This study was supported by TÜBİTAK, TURKEY (grant numbers: 108S124 and SBAG-1774). First three authors contributed equally to this study.

#### P10.22

#### The first case of a genetically proven Maple syrup urine disease in Bulgaria

I. Yordanova<sup>1</sup>, H. Kadam<sup>2</sup>, T. Todorov<sup>1</sup>, R. Tincheva<sup>2</sup>, A. Todorova<sup>1</sup>;

<sup>1</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria, <sup>2</sup>University Pediatric Hospital, Medical University Sofia, Sofia, Bulgaria.

Maple syrup urine disease (MSUD) is a rare autosomal recessive disorder of branched-chain amino acid (BCAA) metabolism caused by dysfunction of the multi-enzyme branched-chain alpha-ketoacid dehydrogenase (BCKDH) complex. BCKDH catalyzes the oxidative decarboxylation of branched-chain alpha-ketoacids produced by transamination of the BCAAs leucine, isoleucine, valine. Classic MSUD is the most common phenotype, represented in about 75% of patients, typically manifested as severe neurological impairment during the neonatal period. Affected infants typically show lethargy, weight loss, metabolic derangements and progressive neurologic signs, like alternating hypotonia and hypertonia. MSUD can be caused by mutations in at least 3 genes: BCKDHA on chromosome 19q13, BCKDHB on chromosome 6q14, and DBT on chromosome 1p21. Mutations in BCKDHA gene were associated with approximately 37% of MSUD cases previously described in the literature. Direct sequencing of the BCKDHA gene was performed in order to screen for germline mutations a dizygotic twin pair with MSUD. The parents are first cousins of Iranian origin. The genetic testing showed one already reported c.452C>T, p.Thr151Met homozygous mutation in only one of the twins. Surprisingly, the second one was negative for this mutation. Both parents were heterozygous carriers. The second child is also MSUD affected as reported by the clinicians. There is a need for further review of clinical data and genetic analysis of the rest two genes to clarify the genetic cause for MSUD in the second twin. We hypothesize the presence of a second genetic change associated with the same clinical picture of MSUD in a single consanguineous family.

#### P10.23

#### Analysis of 37 / 65 muscle genes in 300 patients with neuromuscular diseases

N. Pluta, G. Emmert, W. Kress, C. R. Müller, S. Rost;

Department of Human Genetics, Würzburg, Germany.

Introduction: Neuromuscular diseases (NMDs) are clinically and genetically highly heterogeneous with more than 300 associated genes known to date making conventional molecular diagnosis challenging and expensive. Hence, we designed a targeted gene panel consisting of 37 (version 1) and 65 (version 2) genes associated with the most common types of muscular dystrophies and myopathies.

Methods: A total of 300 patients was analysed using the software GenesearchNGS (PhenoSystems) after target enrichment of the selected muscle genes and next generation sequencing on a MiSeq desktop sequencer (Illumina). About 99% of all coding exons were covered >20x, the overall average coverage was >500x.

Results: After removing recurrent sequencing artefacts and common variants (MAF>2%), we detected more than 4000 variants (~1400 different ones) in the 300 patients analyzed, which we classified from benign to pathogenic (class 1-5). Among these were 35% missense, 29% intronic (9-20 bp flanking sequence), 24% synonymous, 7% potential/essential splice, 4% small deletions/insertions and less than 1% nonsense variants. About 70% of the detected variants were classified as benign (~2200) or likely benign

(~720), approx. 20% as uncertain (~820) and 3-4% as likely pathogenic or pathogenic each (~150 each).

Conclusions: At least one likely pathogenic or pathogenic variant was identified in 180 of the 300 patients suffering from different myopathies (detection rate: 60%). Furthermore, in almost all 300 patients analyzed by our muscle gene panel at least one variant of uncertain significance (class 3) was detected which could be regarded as potentially associated with the myopathy.

#### P10.24

#### Novel recessive mutations in MYH2 presenting with congenital ophthalmoplegia and bulbar palsy

M. Bertoli<sup>1</sup>, M. McEntagart<sup>2</sup>, T. Kerr<sup>3</sup>, V. Straub<sup>1</sup>;

<sup>1</sup>The John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases at Newcastle, Newcastle upon Tyne, United Kingdom, <sup>2</sup>Medical Genetics, St George's University Hospitals, NHS Foundation Trust, London, United Kingdom,

<sup>3</sup>Department of Paediatric Neurology, St Georges University Hospitals NHS Foundation Trust, London, United Kingdom.

Heredity myosin myopathies are a group of clinically heterogeneous muscle diseases, caused by mutations in the skeletal muscle myosin heavy chain (MyHC) genes. Mutations in the MYH2 gene (Myosin heavy chain IIa) have been associated both with an autosomal dominant and a recessive form of proximal myopathy with ophthalmoplegia (MYPOP).

We describe two sisters, aged 5 1/2 and 3 years, who presented at birth with partial external ophthalmoplegia and inability to swallow, requiring gastrostomy feeding. The older sister walked by 2 years of age and often complains about leg pain. She had a normal brain MRI and an EMG showed evidence of a bulbar palsy. The younger sister walked before she was 1 year of age. They both show mild proximal weakness. They can walk independently up to 5 minutes and then need to be carried. They fall frequently and have difficulty climbing stairs. Both sisters share the same compound heterozygous novel mutations in the MYH2 gene (p.S294L and p.Q291H), which were identified through exome sequencing via the Complementary Analysis Project on congenital myopathies within the Deciphering Developmental Disorders (DDD) project.

Bulbar involvement has been described in autosomal dominant MYH2 myopathies in a patient presenting in his teens with proximal weakness who consecutively developed ophthalmoplegia and swallowing difficulties, and in a baby presenting with generalised hypotonia and swallowing difficulties at birth. Here we describe the first case of an MYH2-related myopathy due to compound heterozygous missense mutations, presenting at birth with bulbar palsy as the predominant feature rather than weakness.

#### P10.25

#### A novel MYH2 mutation in family members presenting with congenital myopathy, ophthalmoplegia and facial weakness

T. Willis<sup>1</sup>, C. Hedberg-Oldfors<sup>2</sup>, Z. Alhaswani<sup>1</sup>, R. Kulshrestha<sup>1</sup>, C. Sewry<sup>1</sup>, A. Oldfors<sup>2</sup>,

<sup>1</sup>Robert Jones and Agnes Hunt Hospital, Oswestry, United Kingdom, <sup>2</sup>University of Gothenburg, Gothenburg, Sweden.

Myosin heavy chain (MyHC) is a major structural component of the striated muscle contractile apparatus. There are several striated muscle MyHC isoforms encoded by different genes. In adult human limb skeletal muscle, there are three major MyHC isoforms: MyHC I (slow/beta-cardiac MyHC, MYH7), is expressed in slow, type 1 muscle fibers; MyHC IIa (MYH2) is expressed in fast, type 2A muscle fibers and MyHC IIx (MYH1) is expressed in fast, type 2B muscle fibers. Some fibers express both MyHC IIa and IIx. The different muscle fiber types differ in their physiological properties.

Hereditary myosin myopathies have emerged as an important group of diseases with variable clinical and morphological expression depending on the mutated isoform and type and location of the mutation. Myosin myopathy with external ophthalmoplegia is associated with mutations in MYH2 and is inherited in both dominant as well as in recessive manner.

We present a family with myopathy with early onset proximal muscle weakness, facial muscle involvement and ophthalmoplegia, affecting a woman, her sister and two of her children. The clinical features and inheritance raised the suspicion of mitochondrial myopathy with maternal or autosomal dominant inheritance. Muscle biopsy demonstrated lack of type 2A muscle fibers and genetic work up demonstrated that the disease was caused by a novel recessive MYH2 mutation: c.1009-1G>A resulting in skipping of exon 12, which is predicted to result in a frame shift and introducing a premature stop codon at position 347 (p.Ser337LeufsX11).

## P10.26

**CLCN1 variants identified in a large North American cohort of myotonia congenita patients: novel variants and pedigrees with atypical segregation patterns**

J. C. Tarleton;

Fullerton Genetics Lab, Mission Health System, Asheville, NC, United States.

Myotonia congenita (MC) is characterized by impaired muscle relaxation after contraction presenting as mild to severe muscle stiffness when initiating movement. Cramping and transitory weakness may occur in some patients. Stiffness is relieved by repetitive contractions of the muscle, the "warm-up" phenomenon. Mutations in CLCN1, encoding the muscle-specific chloride channel, ClC1, disrupt channel forming homodimers by a variety of mechanisms resulting in MC. Over 200 variants are recorded in HGMD. The recessive form (Becker disease, OMIM 255700) is usually associated with more severe symptoms than the dominant form (Thomsen disease, OMIM 160800). However, numerous examples are known of CLCN1 variants that segregate as dominant in some families yet as recessive in others. Wide phenotypic variation occurs even among individuals with the same mutation suggesting reduced penetrance and genetic modifier effects are common. Reported here is the compilation of over 10 years of CLCN1 sequence analysis on nearly 500 probands and follow up studies of North American MC families. Thirty five previously unreported variants were identified. During segregation analysis of some MC families we found carriers of variants previously classified as recessive had mild myotonia suggesting semi-dominant and reduced penetrance inheritance. Discussed here are two specific examples, one from a family segregating a presumed loss-of-function splice variant and the second from a missense variant. Both have long been classified as recessive. These studies suggest that haplotype insufficiency may sometimes occur in heterozygotes carrying loss-of-function variants and some heretofore recessive missense variants have mild dominant-negative effects.

## P10.27

**Mutation screening of two presumable Myotonia congenita type Becker endemic regions in Bulgaria**

S. Tincheva<sup>1,2</sup>, B. Georgieva<sup>1</sup>, A. Todorova<sup>1,2</sup>, T. Todorov<sup>2</sup>, I. Litvinenko<sup>3</sup>, V. Mitev<sup>4</sup>;  
<sup>1</sup>Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria, <sup>3</sup>Department of Neurology, University Pediatric Hospital, Medical University, Sofia, Bulgaria.

Myotonia congenita type Becker is an autosomal recessive nondystrophic skeletal muscle disorder, caused by mutations in the CLCN1 gene, encoding skeletal muscle chloride channel-1. The disease is characterized by muscle stiffness and an inability of the muscle to relax after voluntary contraction. It affects primarily the lower limb muscles and later progress to the arms, neck, and facial muscles.

Here we report the results from mutation screening of two presumable endemic regions for CLCN1 mutations in Bulgaria. Previous studies of our group discovered a large family from Bulgarian origin populating a village located in the northwest part of the country. The pedigree showed a number of Myotonia congenita type Becker affected individuals both in vertical and horizontal direction, all of them caring a homozygous missense substitution p.Tyr524Cys. Endogamous marriages are very unusual for the Bulgarian population, supposing a high carrier frequency in this subpopulation. Screening of 154 residents of the corresponding region showed a significant carrier frequency for the p.Tyr524Cys mutation of about 0.65% (1/154). The second interesting region in the context of Myotonia congenita type Becker is the southwest part of the country, where we found a large family from Bulgarian Turkish origin. The disease causing missense mutation p.Val273Met was again present in homozygous state. Surprisingly, the genetic testing of newborns from southwest Bulgaria showed an even higher carrier status of about 2.6% (3/116), disproving our initial hypothesis of endogamous marriages (traditionally common in this subpopulation) being the cause for the disease in these patients.

## P10.28

**Becker myotonia: novel mutations in patients born to consanguineous parents**

I. Sahin, H. B. Erdem, S. Tasdemir, A. Tatar;

Ataturk University Department of Medical Genetics, Erzurum, Turkey.

Introduction: Myotonia congenital (MC) is an inherited muscle disease characterized by impaired muscle relaxation after contraction, resulting in muscle stiffness, present from childhood. All striated muscle groups may be involved. MC is due to Cl<sup>-</sup> channel mutations that reduce the stabilizing Cl<sup>-</sup> conductance and is caused by mutations in the CLCN1 gene. CLCN1 is located on chromosome 7q35 and encompasses 35 kb in genomic DNA with

23 exons. The disease can be inherited either as an autosomal dominant (Thomsen's myotonia, MIM 160800) or an autosomal recessive (Becker's myotonia, recessive generalized myotonia, RGM, MIM 255700) trait. Materials and Methods: We describe three patients from two different healthy consanguineous Turkish family with muscle stiffness and easy fatigability. EMG showed myotonic discharges in all the patients and CK levels were slightly increased in patient 1 and 2 and elevated in patient 3. Genetic investigation has been performed.

Results: Mutation analyses showed a homozygous p.Leu159Cysfs\*11 (c.475delC) mutation in patient 1 and a homozygous p.Y150\* (c.450C>A) mutation in patient 2 and 3 in the CLCN1 gene. These mutations have never been reported before and in silico analyses showed the mutations as disease causing.

Conclusion: The two new CLCN1 variants can be added to the growing database of MC-associated mutations. Our data expand the spectrum of CLCN1 mutations and provide insights for genotype-phenotype correlations of myotonia congenita.

Detailed clinical features of subjects with novel CLCN1 mutations.			
	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Age at examination (yr)	9 yr	14 yr	18 yr
Age at onset (yr)	6 yr	9 yr	10 yr
Family history	-	brother affected	brother affected
Consanguinity	+	+	+
Inheritance	autosomal recessive	autosomal recessive	autosomal recessive
Symptom at onset	lower limb stiffness	lower limb stiffness	lower limb stiffness
Warm-up	+	+	+
Muscle hypertrophy	-	+	-
Muscle pain	+	+	+
Transient weakness	+	+	+
Permanent weakness	+	+	+
Electromyography	Myotonic discharges	Myotonic discharges	Myotonic discharges
Mutation in CLCN1	p.Leu159Cysfs*11 (c.475delC) Homozygous	p.Y150* (c.450C>A) Homozygous	p.Y150* (c.450C>A) Homozygous
Exon/intron of mutations	Exon 4	Exon 4	Exon 4
Diagnosis	Becker Myotonia	Becker Myotonia	Becker Myotonia

## P10.30

**Nemaline myopathy may be caused by a variety of large copy number variations in NEB detectable with the NM-CGH array**K. Kiiski<sup>1</sup>, V. Lehtokari<sup>1</sup>, L. Sagath<sup>1</sup>, C. Wallgren-Pettersson<sup>1</sup>, K. Pelin<sup>1,2</sup>,

<sup>1</sup>The Folkhälsan Institute of Genetics and The Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland, <sup>2</sup>Department of Biosciences, Division of Genetics, University of Helsinki, Helsinki, Finland.

Our custom designed NM-CGH microarray detects copy number variants (CNVs) in the currently known ten nemaline myopathy -causing (NM) genes and one unpublished gene. NM constitutes a heterogeneous group of disorders among the congenital myopathies. Variants in the nebulin gene (NEB) are the most frequent cause for NM. NEB consists of 249kb of genomic sequence including 183 exons. Using the NM-CGH array, 315 samples from 230 families have been analysed to date, and a pathogenic CNV has been identified altogether in 14% of the studied NM families. These include 15 different large disease-causing aberrations in NEB in 31 different families. 12 aberrations were identified in only one family each, one aberration in two families, one in three families and one recurrent aberration in 36 families. The size of the aberrations vary greatly, covering from only a part of one NEB exon (72 bp) to more than half of the gene (133 kb). The recurrent CNV consisting of the 32kb NEB triplicate region (TRI), where eight exons are normally repeated three times, was shown to contain a CNV in 16% of the NM families and in 6% of the families the NEB TRI CNV was interpreted to be pathogenic. Furthermore, one pathogenic CNV (17-21kb) was also identified in another NM gene, TPM3. We are currently updating the NM-CGH microarray to include a larger variety of myopathy causing genes to allow for analysis for the patients with NM-related clinical phenotypes. The NM-CGH microarray method is available for mutation analysis in our laboratory.

## P10.31

**A mutation in EPT1 causes neurodevelopmental delay and spastic paraplegia: a First report of impaired Kennedy pathway**

A. H. Alkhayat AlShehi<sup>1</sup>, F. A. Alsalmi<sup>1</sup>, F. Almurshed<sup>1</sup>, A. AlFutaisi<sup>1</sup>, M. Y. Ahmed<sup>2</sup>, B. A. Chioza<sup>2</sup>, P. Fernandez-Murray<sup>3</sup>, A. Cazenave-Gassiot<sup>4</sup>, M. R. Wenk<sup>5</sup>, M. A. Patton<sup>2</sup>, D. L. Silver<sup>6</sup>, E. Baple<sup>6</sup>, C. R. McMaster<sup>7</sup>, S. Alzuhaibi<sup>1</sup>, A. H. Crosby<sup>8</sup>,

<sup>1</sup>Sultan Qaboos University, Muscat, Oman, <sup>2</sup>University of Exeter, Exeter, United Kingdom,

<sup>3</sup>Dalhousie University, Halifax, NS, Canada, <sup>4</sup>National University of Singapore, Singapore, Singapore, <sup>5</sup>Duke-NUS, Singapore, Singapore, <sup>6</sup>University of Exeter, Exeter, United Kingdom.

Many subtypes of hereditary spastic paraplegia (HSP), a highly heteroge-

neous group of neurodegenerative motor neuron disorders, has been associated with mutations in genes involved in lipid metabolism. Here, we report a new form of autosomal recessive complex HSP associated with mutation in the ethanolaminephosphotransferase 1 (EPT1) gene, responsible for the final step in Kennedy pathway forming phosphatidylethanolamine (PE) from CDP-ethanolamine. PE is a glycerophospholipid that, together with phosphatidylcholine (PC), constitutes more than half of the total phospholipids in eukaryotic cell membranes. We determined that the mutation defined dramatically reduces the enzymatic activity of EPT1, thereby hindering the final step in PE synthesis. Additionally, due to CNS inaccessibility we undertook quantification of PE levels and species in patient and control blood samples as an indication of liver PE biosynthesis. Although this revealed alteration to levels of specific PE fatty acyl species in patients, overall PE levels were broadly unaffected indicating that in blood EPT1 inactivity may be compensated for in part via alternate biochemical pathways. These studies define the first human disorder arising due defective CDP-ethanolamine pathway biosynthesis and provide new insight into the role of Kennedy pathway components in human neurological function.

This study is supported by funding from the MRC (G1002279, G1001931), Newlife, The Research Council, Oman (ORG/HSS/09/002), Genome Canada, Genome Atlantic, the Nova Scotia Research Innovation Trust, the Nova Scotia Department of Health and Wellness to CRM, and the Singapore Ministry of Health's National Medical Research Council (CBRG/069/2014).

#### P10.32

##### A genotype-first approach to defining a neuromuscular disease

*A. Rodríguez<sup>1</sup>, M. Miramar<sup>1</sup>, S. Izquierdo<sup>1</sup>, M. Alcaine<sup>1</sup>, A. Lasierro<sup>1</sup>, E. Barrio<sup>2</sup>, J. González<sup>1</sup>, J. Capabro<sup>1</sup>, E. Mayayo<sup>1</sup>,*

<sup>1</sup>H.U.Miguel Servet, Zaragoza, Spain, <sup>2</sup>Universidad de Zaragoza, Zaragoza, Spain.

**Introduction:** Medical genetics typically entails the detailed characterization of a patient's phenotypes followed by genotyping to discover the responsible gene or mutation. The genetic study of polygenic or similar clinical manifestations diseases, such as neuromuscular, has historically been difficult, meeting with limited success. This difficulty are progressing to a point where a reverse strategy may be fruitful in assigning the pathogenic effects of many different genes and in determining whether particular genotypes manifest as clinically recognizable phenotypes. The aim of this work is to demonstrate the diagnostic throughput of a genetic study by massive exome sequencing in order to identify variants associated with congenital myasthenia disorders of glycosylation channelopathies and neuromuscular diseases.

**Material and Methods:** A 41 years old male patient from neurology comes to our section of clinical genetics with distal myopathy and clinical diagnosis of dysferlinopathy. He's never run and jump well. He notices decreased muscle mass in both twins and difficulty walking fast and misalignment of both feet.

He suffers from spasms in lower extremities to small efforts. After written consent, peripheral blood sample were send to study dysferlin gene. No mutation was found. New sample were sent to an external laboratory (Nimgenetics) and masive exome sequencing were done (199 genes)

**Results:** Homozygous pathogenic variant c.7447A>G (p.Lys2483Glu) in the COL6A3 gene were identified. It's Bethlem myopathy associated. Both parents are carriers.

**Conclusions:** With sequencing becoming increasingly cheaper as well as the preferred frontline diagnostic test, genetics becomes the first step to give the clinical diagnosis of neuromuscular diseases.

#### P10.33

##### Clinical spectrum of mitochondrial diseases associated with POLG1, POLG2 and C10orf2 mutations

*A. Paramasivam<sup>1</sup>, A. K. Meena<sup>2</sup>, M. S. Uppin<sup>2</sup>, K. Thangaraj<sup>1</sup>,*

<sup>1</sup>CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India, <sup>2</sup>Department of Neurology, Nizam's Institute of Medical Sciences, Hyderabad, India.

**Introduction:** Mitochondrial diseases are a heterogeneous group of disorders caused by genetic defects in mitochondrial DNA or in nuclear genes. Mutations in nuclear genes POLG1, POLG2 and C10orf2 required to replicate the mitochondrial genome are reported to affect mtDNA stability such as accumulation of point mutations, multiple deletions and depletion of mtDNA, and cause progressive mitochondrial disorder.

**Materials and Methods:** we sequenced the exons and intron-exon boundaries of the POLG1, POLG2 and C10orf2 genes from >500 unrelated patients with a heterogeneous array of clinical presentations (Myopathy, CPEO, Ataxia, Progressive myoclonus epilepsy, LHON, Neuropathy and ataxia, Leigh syndrome, Leukodystrophy-ataxia-neuropathy etc.) and 2400 population samples from various parts of India were also used to determine the carrier frequencies of common pathogenic mutations in POLG1 (p.A467T, G848S,

W748S).

**Results:** We identified several novel and reported non-synonymous pathogenic mutations in the POLG1, POLG2 and C10orf2. We also identified several synonymous variants and variants in non coding regions. Pathogenicity of novel mutations were assessed based upon (a) absence in 100 normal controls, (b) the mutation causes substitution in conserved amino acid residues of the proteins, (c) location in protein regions of structural/functional importance, (d) co-segregation of the mutations with the disease phenotype and (e) mtDNA multiple deletions and/or depletion. Carrier frequency of common POLG1 mutations is 0.08% for A467T, 0.20% for W748 and 0.00% for G848S in India.

**Conclusion:** Identification of POLG1, POLG2 and C10orf2 mutations is very important for diagnosis, proper medical management and appropriate genetic counseling.

#### P10.34

##### Neutral Lipid Storage Disease with Myopathy: first report of an Italian patient with novel PNPLA2 mutations causing an in frame skipping of exon 5

*S. Missaglia<sup>1</sup>, M. Mora<sup>2</sup>, L. Morandi<sup>2</sup>, D. Cassandrini<sup>2</sup>, F. M. Santorelli<sup>3</sup>, L. Maggi<sup>2</sup>, D. Taviani<sup>1</sup>,*

<sup>1</sup>Catholic University of the Sacred Heart, Milan, Italy, <sup>2</sup>Fondazione IRCCS Istituto Neurologico „Carlo Besta“ Milan, Italy, <sup>3</sup>IRCCS Stella Maris, Pisa, Italy.

Neutral Lipid Storage Disease with Myopathy (NLSDM), is a rare autosomal recessive disorder characterized by excessive lipid accumulation into cytoplasmic lipid droplets present in many tissues, including skin, bone marrow, heart, liver and muscles. Clinically, NLSDM patients present with skeletal muscle myopathy and sometimes with severe dilated cardiomyopathy, hepatomegaly and insulin resistance. NLSDM is caused by a defect in the PNPLA2 gene, which encodes the adipose triglyceride lipase (ATGL), an enzyme that hydrolyses fatty acids from triacylglycerol. Here we report the clinical and genetic findings of an Italian patient carrying two novel PNPLA2 mutations (c.696+4A>G and c.553\_565delGTCCCCCTTCTCG). The patient presented at the age of 39 years with right upper limb abduction weakness progressing slowly over the years with asymmetric involvement of proximal muscle in upper and lower limbs. At the age of 53 mild left ventricular diastolic dysfunction was detected. This is the first report of a NLSDM patient with a splice site mutation causing an in-frame exon 5 PNPLA2 skipping. Molecular analysis revealed that only one type of PNPLA2 transcript, lacking exon 5, was present in patient's cells. Such aberrant mRNA should cause the production of a ATGL protein able to bind lipid droplets but lacking part of catalytic domain. This is a very interesting case displaying severe PNPLA2 mutations with a clinical presentation ranging from slight cardiac impairment to full expression of a severe asymmetric myopathy. This work was supported by Telethon grant (GGP14066). The authors acknowledge Euro-BioBank and the Telethon Network of Genetic Biobanks (GTB12001F), for providing biological samples.

#### P10.35

##### Mutation detection in a patient with muscular dystrophy using a next generation sequencing panel

*H. A. van Duyvenvoorde<sup>1</sup>, R. F. M. de Coo<sup>2</sup>, L. Vijfhuizen<sup>1</sup>, W. S. Frankhuizen<sup>1</sup>, I. B. Ginjaar<sup>4</sup>, S. H. B. Bollen<sup>1</sup>, N. van der Stoep<sup>1</sup>,*

<sup>1</sup>Leiden University Medical Center, Department of Clinical Genetics, Leiden, Netherlands,

<sup>2</sup>Erasmus Medical Center, Department of Child Neurology, Rotterdam, Netherlands.

##### Introduction:

Limb-girdle muscular dystrophy is a clinically and genetically heterogeneous group of muscular dystrophies. Identification of the specific gene defect can be challenging. Immunohistochemistry testing of muscle biopsies can be helpful to select the confirmatory genetic test. Alternatively, direct testing a LGMD genepanel can help identify the causal gene. We describe a boy with severe childhood onset of LGMD with increasing and especially distal weakness of the muscles. Initial investigation on a muscle biopsy showed moderate reduction of dystrophin and dysferlin. However, no DMD gene mutation was identified. Additional investigations provided no strong indication for a dysferlinopathy or sarcoglycanopathy. Previously, several other genes were tested but no causal gene was identified. Therefore we applied our NGS approach that evaluates 39 genes known to be responsible for various Limb-Girdle muscular dystrophies and several muscular myopathy related diseases.

**Materials en Methods:** All coding exons and at least 20 bp of flanking intronic sequences were enriched by using the Agilent SureSelectXT Inherited Disease Panel, followed by sequencing on a Illumina HiSeq2000. Data analysis was performed using a home-made pipeline.

**Results:** High-quality sequence data was obtained. Variant analysis using a low stringency analysis pipeline revealed a homozygous pathogenic frameshift mutation in exon 6 of the SGCG gene.

**Conclusions:** Although investigation of a muscle biopsy did not clearly indicate the involvement of sarcoglycans, we identified a homozygous pathogenic SGCG gene mutation, establishing the diagnosis LGMD2C. Indicating that a broad high-quality NGS genepanel can identify the causal mutation, if muscle biopsy investigation is not conclusive.

#### P10.36

#### Impact of PMP22 duplication on integrins expression in Charcot-Marie-Tooth (CMT1A) transgenic rat model

**M. Jouaud<sup>1</sup>, L. Richard<sup>1</sup>, B. Funalot<sup>2</sup>, F. G. Sturtz<sup>1</sup>, A. Lia<sup>1</sup>;**

<sup>1</sup>Univ. Limoges, Maintenance myélinique et neuropathies périphériques, EA 6309, Limoges, France, <sup>2</sup>Univ. Paris-Est Créteil, Limoges, France.

Charcot-Marie-Tooth disease is the most prevalent hereditary peripheral neuropathy (1/2500). The most common form (CMT1A) is caused by duplication on Peripheral Myelin Protein 22 (PMP22), leading to peripheral progressive demyelination, associated with sensibility and mobility loss of upper and lower limbs. The role of PMP22 is currently not well established. To better understand its role, Amici et al. (2006) created a Pmp22 knockout murine model and observed a reduced expression for some integrins. These integrins ( $\alpha 6\beta 4$  &  $\alpha 6\beta 1$ ) are known to be involved in Schwann cells differentiation and myelination. In addition, a CMT1A-transgenic rat strain, carrying supplementary copies of the Pmp22 gene, was created by Sereda et al. (1996). Our goal was to study in this last model the effect of Pmp22 gene duplication on integrins expression.

At various age (from 1 month to 1 year), we studied, on homozygous, heterozygous and wild-type rats sciatic nerves, PMP22 and integrins expression by quantitative Real-Time-PCR, Western-Blotting, immunohistology and electron-microscopy. Our first results show that some of these integrins are not expressed appropriately. In homozygous and heterozygous rats, we observed mislocation of the PMP22 protein and integrins in the Schwann cells. Interestingly, integrins remain collocated with PMP22 despite its bad location. The gene expression and quantity of protein are also varying in correlation with animal's phenotype and nerve pathology.

These experiments show that the Pmp22 overexpression has a potential role on the Schwann cells differentiation and myelination through interactions with integrins and may explain clinical symptoms of CMT1A patients.

#### P10.38

#### Molecular diagnosis approach based on selection of candidate genes by prioritization scripts. A case of two affected siblings presenting multi-minicore and central core disease respectively.

**S. Carmona<sup>1</sup>, C. Sánchez<sup>1</sup>, J. Lezana<sup>1</sup>, I. Diez<sup>1</sup>, C. Torres<sup>1</sup>, M. Ruíz<sup>1</sup>, J. López<sup>1</sup>, S. Franco<sup>2</sup>, C. Benito<sup>2</sup>, J. López-Siles<sup>1</sup>;**

<sup>1</sup>M.G.C. Genetaq, Málaga, Spain, <sup>2</sup>Fetal-Maternal Hospital, Málaga, Spain.

**Introduction:** Multi-minicore (MmD) and central-core (CCD) diseases are two disorders where RYR1 gene plays a crucial role. Most CCD is associated with mutations in RYR1 whereas MmD is related with pathogenic variants in SEPN1 and RYR1. We report a 5-years-old male patient histologically diagnosed with MmD (patient-1), and his 10-years-old brother diagnosed with CCD (patient-2).

**Materials and Methods:** TruSight-One sequencing panel (Illumina) was carried out and SEPN1 and RYR1 genes were analysed. The variants were generated by alignment against reference genome (UCSC hg19) with BWA aligner and GATK variant caller. Then, three VCF files containing common variants in patients and unique variants were generated using BedTools, and a custom prioritization script was used to select candidate genes based on different phenotype-genotype association databases (Human Phenotype Ontology, DisGeNet, HGMD).

**Results:** Two variants of clinical interest in heterozygous state were detected in RYR1 only in patient-1 (c.6721C>T:p.Arg2241Stop and c.2122G>A:p.Asp708Asn).

As symptoms of patients could not be explained by these results, we decided to search potential causal variants in other candidate genes selected. A variant of potential interest was detected in TTN in heterozygous state in both patients (c.59866\_59869delAGTG:p.Ser19956LeufsX28). Another variant in TTN canonical splice site in heterozygous state (c.15983-1G>A) was detected in patient-1.

**Conclusions:** These variants in TTN gene do not clearly explain the phenotype of patient-2, but could justify the clinical features of patient-1. We keep analysing variants in other potentially related genes by exome sequencing using the approach described, as scripts are easily scalable.

#### P10.39

#### LGMD2D intrafamilial clinical heterogeneity caused by alternative splicing of SGCA gene

**L. Gonzalez-Quereda<sup>1</sup>, J. Diaz- Manera<sup>2</sup>, M. Rodriguez<sup>3</sup>, E. Gallardo<sup>4</sup>, C. Lleixa<sup>2</sup>, P. Piñol-Jurado<sup>5</sup>, V. Straub<sup>6</sup>, A. Topf<sup>6</sup>, P. Gallano<sup>1</sup>;**

<sup>1</sup>Genetics Dept. Hospital de la Santa Creu i Sant Pau, U-705 CIBERER, Barcelona, Spain,

<sup>2</sup>Neurology Dept. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>Genetics Dept. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>4</sup>Neurology Dept. Hospital de la Santa Creu i Sant Pau, CIBERER, Barcelona, Spain, <sup>5</sup>Neurology Dept. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>6</sup>John Walton Muscular Dystrophy Research Centre, Newcastle, United Kingdom.

**BACKGROUND:** Mutations in the SGCA gene cause limb girdle muscular dystrophy type 2D (LGMD2D), a recessive form of muscular dystrophy mainly affecting proximal muscles. Most individuals present onset in childhood with a progressive and severe clinical course while others have first symptoms at young/adult age and a mild form of the disease.

**METHODS:** We report a LGMD2D family with three affected siblings. The index case developed muscle weakness involving paraspinal muscles, showing a dystrophic pattern in the muscle biopsy, considered as an axial myopathy. Her two siblings only had hyperkemia. Exome sequencing was performed as part of Myo-Seq project. Sanger sequencing verified the presence of the variants detected. mRNA isolated from muscle biopsy was analysed and the level of different transcripts quantified.

**RESULTS:** Exome sequencing revealed an intronic deletion located at c.585-31\_585-24 of SGCA in homozygous state. mRNA showed the presence of three different transcripts: 1) The wild type, 2) A transcript with a frameshift deletion of exon 6, and 3) A transcript with a cryptic splicing acceptor leading to a cDNA including 26 extra amino acids coming from intron 6. We confirmed a deficiency of alpha-sarcoglycan in the muscle biopsy using IF and WB. The remaining sarcoglycan complex proteins and dystrophin were all normal.

**DISCUSSION:** The mild clinical presentation of LGMD2D in this family is attributable to the presence of wild type transcript in spite of having a homozygous mutation. The proportion of each transcript can be the key of the different degrees of severity between family members.

#### P10.40

#### X-linked spastic paraparesia with dysarthria caused by insertional translocation into the Xq26.3 located inverted repeat

**\*. Asan<sup>1</sup>, T. Thelle<sup>2</sup>, R. Christensen<sup>3</sup>, L. Bolund<sup>4</sup>, A. Gunnarsson<sup>3</sup>, Z. Quan<sup>1</sup>, X. Zhang<sup>1</sup>, J. Li<sup>5</sup>, P. Qvist<sup>4</sup>, A. Børglum<sup>4</sup>, E. Kamsteeg<sup>6</sup>, J. Hedegaard<sup>3</sup>, B. Peters<sup>7</sup>, L. Sunde<sup>2</sup>, M. Nyegaard<sup>4</sup>, U. Jensen<sup>3</sup>,**

<sup>1</sup>BGI-Shenzhen, Shenzhen 518083, China, <sup>2</sup>Regionshospitalet Viborg, Viborg, Denmark,

<sup>3</sup>Aarhus University Hospital, Aarhus N, Denmark, <sup>4</sup>Aarhus University, Aarhus C, Denmark,

<sup>5</sup>Southeast University, Nanjing, China, <sup>6</sup>Radboud UMC, Nijmegen, Netherlands, <sup>7</sup>Complete Genomics, Mountain View, CA, United States.

**Background and aim:** Spastic paraparesia is a genetically heterogeneous disease that can be caused by alterations in one of many loci. We describe a family with X-linked complicated spastic paraparesia with early onset dysarthria. Mild intellectual disability is described in some patients.

**Methods:** In order to pinpoint candidate gene, we used a combination of linkage analysis, exome sequencing and whole genome sequencing. Linkage analysis showed linkage to a region within Xq26.3 containing 52 genes. As the exome sequencing did not reveal any pathogenic mutations we used the Complete Genomics platform to sequence the entire genome of one patient and one healthy male control. RNA-seq of PAX blood, fibroblasts, iPSC and neuroprogenitor cells were quantified.

**Results:** Whole genome sequencing detected an insertional translocation of chromosome 4 material into a palindromic inverted repeat located within Xq26. Such translocation has been described before resulting in both XD hypertrichosis and XR hypertrichosis. XR CMT phenotype has also been described as well as hypoparathyroidism. To pinpoint the disruption in gene expression, we analysed the expression of the entire transcriptome and found that the activity of multiple genes was affected.

**Conclusion:** It is very likely that such insertions changes the topologically associated domains (TADs) and causes a rewiring of the region. However, our results also illustrate the difficulties in pinpointing a single candidate gene. As we suspect that some of the differentially expressed genes are due to normal variation between case and control we will discuss a strategy to narrow down the number of candidate genes.

## P10.41

### Novel truncating mutation is attributed to Spastic Paraplegia-64

M. Noruzinia<sup>1,2</sup>, O. Bashti<sup>1</sup>, M. Ahmadvand<sup>3</sup>, A. Salehi Chaleshtori<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of, <sup>2</sup>Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran, Islamic Republic of, <sup>3</sup>Department of Hematology and Blood Banking, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of.

**Introduction:** Neurodegenerative genetic diseases of motor neuron characterized by progressive age-dependent defeat of corticospinal motor tract function celebrated as Hereditary Spastic Paraplegias (HSPs). Main features are axonal degeneration and progressive lower limb spasticity. One of them, Spastic Paraplegia-64 (SPG64; OMIM# 615683) is an autosomal recessive paradigm, established by homozygous or compound heterozygous mutations in the ENTPD1 gene.

**Materials and Methods:** A seven years old male with chief compliments of severe neurodevelopmental delay referred to SCRC in order to genetic testing. Patient's Blood collected from antecubital veins, genomic DNA extracted and applied to exome capture, library generation and exome sequencing. Then inheritance validation of candidate variants confirmed by the Sanger sequencing in all family members.

**Results:** A homozygous single base pair deletion in exon 8 of the ENTPD1 gene (chr10:9762261delA) that drives a frameshifting and termination of the protein two amino acids downstream to codon 383 (p.Asn383ThrfsTer2; ENST00000371207) was detected. No other variant that warrants to be reported was detected. This frameshift variation is not reported in both 1000 genomes and ExAC databases.

**Conclusion:** This mutation led to NMD, frameshifting and production of truncated protein. This mutation truncate the ENTPD1 protein in its extracellular domain partially and its helical and cytoplasmic domains entirely. We concluded that this truncated protein is the most logical causative for explanation of the SPG-64 phenotype in reported patient and this mutation would be applied in PND and PGD in the family. This study was funded by SCRC of Sarem women's hospital.

## P10.42

### DYNC1H1 gene methylation correlates with a severity of spinal muscular atrophy

M. A. Maretina<sup>1,2</sup>, G. Y. Zheleznyakova<sup>3</sup>, V. S. Baranov<sup>1,2</sup>, A. V. Kiselev<sup>1</sup>;

<sup>1</sup>D.O. Ott Research Institute of Obstetrics and Gynecology RAMS, Saint-Petersburg, Russian Federation, <sup>2</sup>Saint-Petersburg State University, Saint-Petersburg, Russian Federation, <sup>3</sup>Karolinska Institute Stockholm, Solna, Sweden.

**Introduction:** Spinal muscular atrophy (SMA) - inherited neuromuscular disorder caused by homozygous mutations of SMN1 gene. Respective of manifestation and achieved motor milestones SMA is subdivided into four clinical types. The number of SMN2 gene copies determines SMA severity. Involvement of some modifiers in SMA progression including DNA methylation is also suspected. Our recent genome-wide methylation study revealed 40 genes with differentially methylated CpG sites in SMA patients compared to healthy subjects. DYNC1H1, SLC23A2 and CDK2AP1 genes picked up in our previous study and most probably participating in SMA progression were chosen for the present work.

**Material and Methods:** Analysis of melting profiles was carried out in 78 DNA samples from SMA patients with severe type I (40) and mild types III-IV (38) of disease. High-resolution melting procedure with bisulphite-treated DNA was used. Methylation level was estimated by interpolating polynomials using values of DNA standards with known percentage of methylation. **Results:** No differences in methylation of 5'-UTR of SLC23A2 and CDK2AP1 as well as exon 41 of DYNC1H1 were found in the patients with severe and mild SMA. Significantly higher methylation of DYNC1H1 exon 37 was registered in mildly affected SMA patients compared to SMA type I patients.

**Conclusion:** Exon 37 of DYNC1H1 lies within CpG-rich region and apparently should be methylated for preventing spurious transcription initiations. Mutations in DYNC1H1 were shown to be associated with common neuromuscular diseases, such as ALS and dominant SMA. Feasible involvement of DYNC1H1 into SMA pathogenesis is suggested.

## P10.43

### Analysis of perineuronal net components in neuronal differentiation defects

N. Eskici<sup>1</sup>, S. Erdem-Ozdamar<sup>2</sup>, D. Dayangac-Erden<sup>1</sup>;

<sup>1</sup>Hacettepe University Faculty of Medicine Department of Medical Biology, Ankara, Turkey, <sup>2</sup>Hacettepe University Faculty of Medicine Department of Neurology, Ankara, Turkey.

**Introduction:** Perineuronal net (PNN) is composed of a specialized extracellular

matrix which is found around neuronal cell body and dendrites in the central nervous system. PNN is responsible from synaptic stabilization in neurons and regulation of plasticity. It also has neuroprotective role against excitotoxicity and oxidative stress. One of the major elements of PNN are hyaluronan and proteoglycan link protein (HAPLN1), tenascin-R (TNR) and aggrecan (ACAN) proteins.

**Materials/Methods:** In order to investigate the effects of HAPLN1, TNR and ACAN during neural differentiation; PC12 cell line which is derived from the neural crest lineage and undergo to a neural phenotype when treated with NGF was used as a differentiation model. mRNA, protein expression levels and distribution of PNN were analyzed before and after differentiation (day 3,5,7). Also neurite length analysis were performed.

**Results:** Our study demonstrated that HAPLN1 and ACAN expression showed an increase at day 7 and the longest neurite were detected at that time point ( $p < 0.05$ ). On the other hand TNR RNA/protein levels remained unchanged. In addition to our results obtained from PC12 model of neuronal differentiation, we used spinal muscular atrophy (SMA) as a disease model which is caused by neuritogenesis defects. Using transcriptome analysis, we identified that extracellular matrix- synapse organization pathways were affected and PNN components showed a significant decrease in SMA fibroblasts. The results were validated and 2 to 5 fold decrease was detected.

**Conclusions:** In-vitro disease models and mouse models will also provide information on PNN dysfunction in the pathogenesis of other neurodegenerative diseases. TÜBİTAK:114S914

## P10.44

### Occurrence of CHCHD10 mutations in Finnish patients with motor neuron disorder

S. Penttilä<sup>1</sup>, M. Jokela<sup>2</sup>, A. Saukkonen<sup>3</sup>, J. Toivanen<sup>2</sup>, B. Udd<sup>1,4,5</sup>;

<sup>1</sup>Neuromuscular Research Unit, University of Tampere, Tampere, Finland, <sup>2</sup>Department of Neurology, Turku University, Turku, Finland, <sup>3</sup>Department of Neurology, Central Hospital of Northern Karelia, Joensuu, Finland, <sup>4</sup>University of Helsinki, Folkhälsoinstitutet och Departament of Medical Genetics, Helsinki, Finland, <sup>5</sup>Department of Neurology, Vaasa Central Hospital, Vaasa, Finland.

**Background and aims:** Spinal muscular atrophy Jokela type (SMAJ, OMIM #615048) is a relatively benign form of motor neuron disease. The disease is caused by a dominant mutation c.197G>T p.G66V in CHCHD10 that is a founder mutation in Finland. Other mutations in CHCHD10 have been reported to cause frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) with mitochondrial myopathy and isolated mitochondrial myopathy. Some mutations have been associated with FTD-ALS but their pathogenicity has remained uncertain. We wanted to know if the mutation c.197G>T p.G66V might cause other phenotypes besides SMAJ. Other aims of the study were to find out if there are other mutations of CHCHD10 in Finnish patients and if CHCHD10 mutations are frequent among patients with distinct neurogenic disorders.

**Methods:** Exon 2 of CHCHD10 that harbors all reported mutations was sequenced in 308 patients. In 103 patients the whole coding region of the gene was sequenced. Of the patients included in the study, 208 patients had a phenotype compatible with SMAJ, 22 patients had a non-specific neurogenic disorder, 14 patients had mitochondrial myopathy, and 64 patients had diagnosis of ALS.

**Results:** The founder mutation was found in 20 patients. All of them were suspected to have SMAJ. No other possibly pathogenic mutations were found, and no mutations were found in other patient groups.

**Discussion and conclusions:** Our results suggest that the CHCHD10 mutation c.197G>T p.G66V is a common mutation in Finland with a prevalence around 2/100000. The clinical outcome of c.197G>T p.G66V is very restricted to the SMAJ phenotype.

## P10.45

### Genetic risk factors associated with Sporadic Inclusion Body Myositis

M. Johari, M. Arumilli, N. Sandholm, H. Lohi, P. Hackman, B. Udd; Folkhälsoinstitutet och Departament of Medical Genetics, Helsinki, Finland.

Sporadic Inclusion Body Myositis (s-IBM) is the most frequent disabling progressive muscle disease of unknown cause in elderly people, onset at 45 years or later, with coexistence of degenerative pathology and inflammatory muscle changes. In Finland, the estimated prevalence is 70/million. Because of the dual pathology, different pathogenic mechanisms are considered; one suggests that multiple post-translationally modified proteins accumulated in the s-IBM aging muscle fibers may be eliciting a T-cell inflammatory reaction. This abnormal proteostasis and muscle fiber injury could be the result of defects in several different susceptibility genes, making s-IBM a complex multifactorial disease. The isolation of Finnish population provides an excel-

lent opportunity to look for possible disease causing associations. By whole exome sequencing (WES) followed by association analysis we have found genetic variants that have an observed considerably higher frequency in 30 Finnish s-IBM patients compared to control population. These variants could individually or in combination be associated with higher susceptibility for the disease. We are currently investigating the effects on these associations with an increased cohort size and wider population. Further, we are going to perform in-silico network and pathway analysis to identify possible connections of the identified candidate genes with regard to molecular mechanisms of muscle homeostasis, autophagy and injury.

#### P10.46

#### Systematic analysis of polymorphic tandem repeats through the development of a NGS method

*L. Corrado<sup>1</sup>, R. Bordini<sup>2</sup>, L. M. Genovese<sup>3</sup>, E. Mangano<sup>2</sup>, F. Geraci<sup>3</sup>, M. Severgnini<sup>2</sup>, R. D'Aurizio<sup>4</sup>, C. Locci<sup>1</sup>, F. De Marchi<sup>5</sup>, L. Mazzini<sup>4</sup>, A. Brusco<sup>5</sup>, G. Manzini<sup>6</sup>, M. Pellegrini<sup>3</sup>, G. De Bellis<sup>2</sup>, S. D'Alfonso<sup>1</sup>;*

<sup>1</sup>Dept. of Health Science, University of Eastern Piedmont UPO, Novara, Italy, <sup>2</sup>Institute for Biomedical Technologies (ITB), National Research Council (CNR), Segrate (MI), Milan, Italy, <sup>3</sup>Institute for Informatics and Telematics (IIT), National Research Council (CNR), Pisa (PI), Pisa, Italy, <sup>4</sup>Dept. of Neurology, Ospedale Maggiore della Carità, Novara, Italy, <sup>5</sup>Department of Medical Science, University of Torino, Italy, Torino, Italy, <sup>6</sup>Institute for Informatics and Telematics (IIT), National Research Council (CNR), Pisa (PI), Department of Science and Technological Innovation University of Piemonte Orientale, UPO, Novara, Italy.

Tandem repeats polymorphisms (TRPs) are extremely common throughout the genome and represent good candidates for missing hereditability in different neurodegenerative diseases for whom the expansion of TRP represents the major disease gene.

To date, TRPs analysis is a remarkable challenge using current next generation sequencing (NGS) approach and thus they were never systematically analyzed at genome wide level.

The aim of this study is to implement a pipeline for the TRPs analysis from genome-wide NGS data. To this purpose, we successfully implemented a new procedure to genotype TRPs, which we applied to a NGS target-resequencing panel containing over 10000 TRP loci, including 37 known disease-causing TRPs.

Fifteen DNA patients affected by nine different neurodegenerative diseases and carrying expanded alleles of different lengths/repeat motif have been sequenced and analyzed together with one CEPH sample with about 9900 known TRPs genotyping.

Our approach correctly determines the allele size of 76% of known genotypes at 63 TR loci in the CEPH sample. Similarly, when considering the typing in known disease-related loci we obtained a consistent genotyping in almost all cases (98% of 56 genotypes). Even for very large expansions such those in c9orf72 and DM, which are indeed not measurable, we identified those samples carrying a pathological expanded allele. Accordingly, our NGS approach can be applied to discover new disease loci characterized by tandem repeat expansion. With this aim, we are performing a comprehensive genome-wide research of TRPs in all gene regions in a large cohort of ALS patients with a WGS approach.

#### P10.47

#### Targeted next-generation sequencing as a diagnostic tool in neuromuscular disorders

*S. Lehtinen<sup>1</sup>, S. Penttilä<sup>1</sup>, T. Suominen<sup>1</sup>, B. Uda<sup>1,2,3</sup>;*

<sup>1</sup>Neuromuscular Research Center, Tampere University and University Hospital, Tampere, Finland, <sup>2</sup>Folkhälsan Institute of Genetics and Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland, <sup>3</sup>Department of Neurology, Vasa Central Hospital, Vasa, Finland.

Diagnosing neuromuscular disorders is often very challenging. There is considerable overlap of clinical findings between different disorders. Furthermore, the genes expressed in muscles can be large and one gene can cause different disorders.

The aim of this study was to evaluate the diagnostic power of our new analytic tool, MYOcap gene panel. It is a next-generation sequencing (NGS) assay targeted to all exons of all known and predicted myopathy or muscular dystrophy causing genes.

The acquired sequencing data was filtered so that only the variants meeting our quality requirements, having a population frequency less than 1 %, and coverage of at least 20X remained. 40 patients were included in this evaluation study. The patients presented with the clinical phenotype of muscular dystrophy or myopathy but the genetic background of their disorder had not been resolved despite of thorough investigations.

With MYOcap, we were able to reach a definitive diagnosis in 12,5 % of the

cases, usually meaning known mutations in genes compatible with the phenotype. 7,5 % of the cases were suspected to have either a new phenotype linked to a known disease-causing gene or a completely new phenotype-genotype correlation. In 20 % of the cases we could clearly state that no variations compatible with the patient's phenotype were found. In 60 % of the cases further studies were recommended.

We conclude that MYOcap is an effective tool for assigning a genetic diagnosis in neuromuscular disorders and can also be used to discover new phenotype-genotype correlations.

#### P10.48

#### Clinical and genetic characterization of the first reported Spanish family with UBQLN2 mutation

*B. Quintáns<sup>1,2</sup>, A. Ordóñez-Ugalde<sup>1,3</sup>, E. Goberna<sup>4</sup>, P. Díaz<sup>1</sup>, S. Zúñiger<sup>5</sup>, J. Martínez-Simón<sup>6</sup>, Á. Carracedo<sup>3,6,2</sup>, M. J. Sobrido<sup>1,6,2</sup>;*

<sup>1</sup>Instituto de Investigación Sanitaria, Santiago de Compostela, Spain, <sup>2</sup>CIBERER, Madrid, Spain, <sup>3</sup>USC, Santiago de Compostela, Spain, <sup>4</sup>Complejo Hospitalario Torrecárdenas, Almería, Spain, <sup>5</sup>University of Miami, Miami, FL, United States, <sup>6</sup>Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain.

Introduction: Mutations in the UBQLN2 gene are the cause of a diversity of neurological syndromes: amyotrophic lateral sclerosis 15 (ALS15) with or without frontotemporal dementia (FTD), and spastic paraparesis (HSP). Spanish patients with UBQLN2 mutations have not been reported so far.

Patients and methods: We studied a large family from Southern Spain in which at least 9 members had a progressive disorder combining upper and, subsequently lower motor neuron manifestations. Clinical data were obtained from 11 individuals in 3 generations. The C9orf72 mutation was analyzed by RP-PCR. Whole exome sequencing (WES) was carried out on the proband. Variants in genes causing HSP, ALS and FTD were prioritized. Results: The C9orf72 expansion was not detected. We identified a previously reported pathogenic variant in UBQLN2 (NM\_013444.3:c.1516C>T;NP\_011630.15:p.P506S) segregating with the disease. There was variability in onset age (generally younger in males), initial symptoms (variable combination of upper and lower motor neuron), severity and progression rate (generally more severe in men, although one affected woman died at 42). Symptoms of FTD were frequent as disease advanced. Penetrance was complete in males and incomplete in females (asymptomatic carriers in the sixth and eighth decades).

Conclusions: UBQLN2 must be ruled out in patients with HSP, even in the absence of second motor neuron signs. The WES approach is useful for the diagnosis of clinically heterogeneous disorders. Our data are in agreement with the phenotype previously described in families with this particular mutation. Genetic counseling is challenging in this X-linked dominant, devastating disease. Funding: ISCIII-FIS PS09/01830.

#### P10.49

#### Activation of the mitochondrial unfolded protein response (UPRmt) pathway in C2C12 myoblast cell line

*E. Aksu<sup>1</sup>, B. Talim<sup>2</sup>, P. Dincer<sup>1</sup>, B. Balcı-Hayta<sup>1</sup>;*

<sup>1</sup>Hacettepe University Faculty of Medicine, Department of Medical Biology, Ankara, Turkey, <sup>2</sup>Hacettepe University Faculty of Medicine, Department of Pediatrics, Pathology Unit, Ankara, Turkey.

Introduction: The mitochondrial unfolded protein response (UPRmt) is a mitochondrial stress response pathway that adapts the protein folding capacity of the organelle to the load of unfolded and misfolded proteins in normal physiology and disease states. Although the detailed mechanism is not known, UPRmt mechanism activates transcription of nuclear-encoded mitochondrial chaperones and quality control proteases to promote protein homeostasis within the organelle. Mitochondrial dysfunction, one of the main causes of muscle degeneration, has been recognized as a key pathological hallmark of several major neuromuscular disorders. However, the relationship between mitochondrial dysfunction and UPRmt has not been investigated yet. For this purpose, an in-vitro model of UPRmt was generated as a continuously available infrastructure for high-throughput analysis. Materials and Methods: UPRmt activation in C2C12 cells was performed by treating cells with an appropriate non-toxic conditions of antimycin (100/150μM), a known inhibitor of mETC complex III, in serum-free media at 37°C for 90 minutes.

Results: Antimycin-induced UPRmt activation was reported by the observation of decreased mitochondrial membrane potential and impaired dynamics as detected by Mitotracker Red-CMXros staining, altered bioenergetic function of mitochondria by MTT assay, high levels of reactive oxygen species measured by DCFDA assay and increased levels of mitochondrial specific chaperone Hsp60 by Western Blot.

Conclusions: The development of an in-vitro model of UPRmt in C2C12

cells will pave the way for identifying the novel genes/proteins involved in UPRmt pathway and novel drug targets by screening libraries of small molecules. This study was supported by TÜBİTAK (Project no:114S876).

#### P10.50

#### Targeted Whole Exome Sequencing analysis allows for identification of a new case of myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK)

R. Szymczak<sup>1</sup>, K. Spodar<sup>1</sup>, A. Wasowska<sup>1</sup>, M. Kania<sup>1</sup>, E. Matczynska<sup>1</sup>, M. Jurkowska<sup>1</sup>, A. Lasek-Bał<sup>2</sup>, A. Potulska-Chromik<sup>3</sup>, A. Kostera-Pruszczyk<sup>3</sup>, A. M. Boguszewska-Chachulsk<sup>1</sup>;

<sup>1</sup>Genomed SA, Warsaw, Poland, <sup>2</sup>High School of Science, Medical University of Silesia in Katowice, Department of Neurology, Katowice, Poland, <sup>3</sup>Department of Neurology, Medical University of Warsaw, Warsaw, Poland.

Whole Exome Sequencing was performed for a seventeen years old male patient with myoclonic jerks, cerebellar ataxia, slight extrapyramidal signs with epileptiform pattern in EEG.

The patient's intellect was normal, he had neither obvious dysmorphic features nor congenital anomalies. CNS imaging and extensive biochemical examinations revealed no apparent abnormalities.

Previous genetic testing of SCA1,2,3, Friedreich ataxia and for a dynamic mutation in the *CSTB* gene, associated with Unverricht-Lundborg disease, gave normal results.

A whole exome sequencing was performed in our laboratory and targeted exome sequencing data analysis has been carried out to identify pathogenic mutations in almost 400 genes associated with: progressive myoclonus epilepsy, spastic paraparesis, ataxia and extrapyramidal signs, and in the mitochondrial genome.

A newly described, recurrent, pathogenic mutation c.959G>A, p.(Arg320His) was identified in one allele of the *KCNC1* gene, which can be considered, with a high probability, to be the main cause of clinical symptoms observed. The carrier status analysis performed in Patients' parents demonstrated that the mutation occurred *de novo*. No pathogenic mutations or potentially pathogenic variants, which could be responsible for the Patient's clinical picture as a whole, have been identified in the other analysed genes.

The result of the analysis support the diagnosis of progressive myoclonus epilepsy type 7, described in the publication of Muona *et al.* (2015) as myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK).

Recently performed targeted neurological examination seems to confirm the MEAK diagnosis.

#### P10.51

#### Clinical, molecular and histopathological findings in a girl with Xq28 duplication resulting in X-linked myotubular myopathy

K. Kosma<sup>1</sup>, M. Tzetis<sup>1</sup>, A. Mitrakos<sup>1</sup>, M. Braoudaki<sup>1</sup>, C. Sofocleous<sup>1</sup>, A. Xaidara<sup>2</sup>, G. Papadimas<sup>3</sup>, A. Tsezuou<sup>4</sup>, S. Kitsiou-Tzeli<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, University of Athens, Athens, Greece, <sup>2</sup>First Pediatric Department, University of Athens, Aghia Sophia Children's Hospital, Athens, Greece,

<sup>3</sup>Department of Neurology, University of Athens, Aeginition, Athens, Greece, <sup>4</sup>Laboratory of Cytogenetics and Molecular Genetics, University of Thessaly, Larissa, Greece.

X-linked myotubular myopathy (XLMTM) is a rare inherited neuromuscular disorder, clinically characterized by severe hypotonia, respiratory failure and skeletal muscle weakness, typical of congenital myopathies, as well as abundant central nuclei as the most prominent histopathological feature on muscle biopsy, thus being grouped in the genetically heterogeneous group of Centronuclear Myopathies (CNMs). XLMTM is associated with mutations and CNVs in the Xq28 chromosomal region, that contains the MTM1 gene. Here we report a case of a severely affected girl with XLMTM, caused by a maternally inherited duplication in the Xq28 region, identified by high resolution aCGH analysis, utilizing the 4x180k aCGH+SNP platform from Agilent Technologies. Furthermore, we studied the methylation profile to determine the X-inactivation pattern that led to the disease and conducted histopathological examination on tissue from muscle biopsy that confirmed the diagnosis of XLMTM. A full clinical examination was also performed. DNA microarray analysis of the peripheral blood sample and muscle biopsy of the proband and her mother revealed a 661kb microduplication in the Xq28 region, containing the MAMLD1, MTM1, MTMR1 and CD99L2 genes. The muscle biopsy revealed histopathological changes usually observed in centronuclear myopathies. To our knowledge this is the first report of a girl with XLMTM caused by Xq28 microduplication. This case report adds to the knowledge on centronuclear myopathies underlying the necessity of thorough genetic testing in the investigation of rare genetic syndromes.

#### P10.52

#### Impact of two SMN1 polymorphisms to improve SMA carriers diagnosis

L. Alias<sup>1,2</sup>, S. Bernal<sup>3</sup>, E. Martínez<sup>3</sup>, F. March<sup>3</sup>, A. Venceslá<sup>4</sup>, M. Baiget<sup>1</sup>, E. Tizzano<sup>5</sup>;  
<sup>1</sup>U705-CIBERER, Barcelona, Spain, <sup>2</sup>Genetics Department. Hospital Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>Genetics Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>4</sup>IRBB-Sant Pau, Barcelona, Spain, <sup>5</sup>Department of Clinical and Molecular Genetics, Hospital Vall d'Hebron, Barcelona, Spain.

Introduction: Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease caused by deletions/mutations in the SMN1 gene. Classical SMA carriers (1/0) with one of their SMN1 copies mutated show a frequency around 1/40 in our population. SMA carriers with two copies of the SMN1 gene in cis have been described. These 2/0 SMA carriers are undistinguishable from non carrier individuals (1/1) by quantitative studies. In the Ashkenazi population, two polymorphisms have been associated with two SMN1 copies in the same chromosome: g.27134G>T in intron 7 and g.27706\_27707delAT in exon 8. Furthermore, these alleles are more frequent in Africans in which the presence of chromosomes with two SMN1 copies is higher. Material: We analyzed these markers in 368 Spanish individuals including SMA patients with SMN2-SMN1 hybrid genes, 1/0 carriers, controls and individuals with more than one SMN1 copy in cis. Methods: Sanger sequencing from the exon 7 to 8 of the SMN1 gene. Results: Both SNP's are more frequently detected in chromosomes with two SMN1 copies in cis in comparison with chromosomes carrying one copy. The g.27706\_27707delAT was more frequent in SMN2-SMN1 hybrids than in wild type SMN1 genes (16.7 versus 1.3%, p<0.05). Conclusion: The impact of these polymorphisms depends on the population studied. In our Spanish population, they are less valuable to predict putative 2/0 carriers and their absence does not exclude the existence of two SMN1 in cis. The high frequency of g.27706\_27707delAT in hybrids genes suggest that chromosomal rearrangements are prone to occur in presence of this polymorphism.

#### P11 Multiple Malformation/anomalies syndromes

#### P11.001

#### Tissue-specific mosaicism of partial 13q monosomy identified in fibroblasts of the patient with mild intellectual disability, dysmorphic features and skin lesions

K. Ziemiakiewicz, M. Smyk, K. Jakubów-Durska, A. Kutkowska-Kaźmierczak, B. A. Nowakowska;

Dep of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.

Introduction: The severity of symptoms in 13q monosomy may vary greatly, depending upon the size and location of the deletion and degree of mosaicism. Large deletions are generally associated with growth delay, mostly severe or moderate mental retardation, defects of the hands and feet, ocular abnormalities, and other major malformations. We describe a girl diagnosed at the age of 2 years 10 months with psychomotor and speech delay, clinodactyly of the 5<sup>th</sup> finger, big hands and feet and very light iris. At the age of 5 karyotype was performed due to clinical features: linear and whirled hypochromic skin lesions, abnormal iris pigmentation and hair texture. At the age of 6 years patient presented mild intellectual disability and subtle dysmorphic features.

Materials and Methods: aCGH analysis was performed using CytoSure ISCA, OGT and chromosome lymphocytes and fibroblasts analysis by G-banding techniques.

Results: In the aCGH analysis no abnormality was found. GTG banding of metaphases from skin fibroblasts showed the 13q deletion in 38 % of cells. The karyotype was: mos 46,XX,del(13)(q12.1)[16]/46,XX[26]. In chromosome analysis of 600 blood lymphocytes metaphases only one abnormal cell with deletion 13q was detected.

Conclusions: Patients with tissue-specific mosaicism may develop much less severe phenotype despite large size of 13q deletion. Karyotyping of skin, to detect mosaicism is warranted in all patients presenting with hypo/hyperpigmentary changes and/or eyes and hair abnormalities regardless of normal peripheral blood aCGH result.

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**P11.002****Three cases of unusual chromosomal rearrangements involving the chromosome 22q11.2 region**

V. Cejnova<sup>1</sup>, V. Harmas<sup>1</sup>, L. Liskova<sup>1</sup>, M. Tothova<sup>2</sup>, P. Hedvicakova<sup>3</sup>, A. Klimova<sup>1</sup>, M. Soukupova<sup>1</sup>, J. Lastuvkova<sup>1</sup>;

<sup>1</sup>Regional Health Corporation, Masaryk Hospital in Usti nad Labem, Department of Medical Genetics, Usti nad Labem, Czech Republic; <sup>2</sup>GENVIA, Prague, Czech Republic;

<sup>3</sup>Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic.

Chromosome 22q11.21 contains a cluster of low-copy repeats (LCRs), referred to as LCR22A-H, that mediate meiotic non-allelic homologous recombination, resulting in either deletion or duplication of various intervals in the region. The deletion of the DiGeorge/velocardiofacial syndrome interval LCR22A-D is the most common recurrent microdeletion in humans, with an estimated incidence of 1: 4,000 births. Although both deletion and duplication events should occur in equal proportions, microduplications of the 22q11.2 region are about half as frequent as microdeletions. Probably, microduplications are underdiagnosed by karyotype analysis and FISH. We present clinical, cytogenetic and molecular analysis of three cases involving the 22q11.2 region. In the first case, we report prenatal diagnosis of a 2.6 Mb deletion in the 22q11.2 region and a 1.7 Mb duplication in the 16p13.11 region by array CGH in a fetus with congenital heart defect. 16p13.11 microduplication syndrome is a recently described syndrome associated with variable clinical features including behavioral abnormalities, developmental delay, congenital heart defects and skeletal anomalies. In the second case, array CGH showed a 156 kb interstitial microdeletion of 22q11.21 in a pregnant women with complex congenital heart disease. The microdeletion affected ten genes, including CRKL, which is considered a candidate critical gene for the 'central' deletions in 22q11.2. In the third case, we detected prenatally a 22q11.2 microduplication of seven probes (HIRA, CLDN5, KIAA1652, KLHL22, PCQAP, SNAP29 and LZTR1) using MLPA. All findings were confirmed by FISH and we also investigated parents in order to determine the parental origin of chromosomal rearrangements.

**P11.003****Cytogenomic delineation of Brazilian patients with 22q11.2 deletion syndrome associated autoimmune heterogeneous phenotype**

C. Milani<sup>1</sup>, F. A. R. Madia<sup>1</sup>, T. V. M. M. Costa<sup>1</sup>, J. G. Damasceno<sup>1</sup>, E. A. Zanardo<sup>1</sup>, A. T. Dias<sup>1</sup>, M. M. Montenegro<sup>1</sup>, G. M. Novo-Filho<sup>1</sup>, F. B. Piazzon<sup>1</sup>, A. M. Nascimento<sup>1</sup>, M. Rocha<sup>2</sup>, D. C. Soares<sup>3</sup>, M. S. Grassi<sup>3</sup>, A. F. L. Carvalho<sup>4</sup>, D. Bertola<sup>3</sup>, M. I. Melaragno<sup>5</sup>, C. A. Kim<sup>3</sup>, M. M. S. Carneiro-Sampaio<sup>3</sup>, L. D. Kulikowski<sup>1,2,3</sup>;

<sup>1</sup>Faculdade de Medicina da USP, São Paulo, Brazil, <sup>2</sup>Centro de Reprodução Humana e Genética, Faculdade de Medicina do ABC, Santo André, Brazil, <sup>3</sup>Unidade de Genética, Departamento de Pediatria, Instituto da Criança, São Paulo, Brazil, <sup>4</sup>Departamento de Biologia Geral, Laboratório de Genética Humana e Mutagenese, Universidade Federal da Bahia, Salvador, Brazil, <sup>5</sup>Departamento de Morfologia e Genética da Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil.

The 22q11.2 Deletion Syndrome phenotype is very heterogeneous with variable expression of the different features including the immunodeficiency. The clinical evidence suggests that the immunodeficiency can be seen in most of the patients with this alteration and the immunological phenotype may vary widely between the patients and the deletion size does not appear to be responsible for this variation. Thus new insights into pathophysiology are important for a better assessment and targeting treatment for the patients.

Cytogenomic alterations in 22q region were investigated using MLPA and SNP-array (HumanCytoSNP-12 BeadChip and CytoSNP-850K BeadChip) to map the presence of Copy Number Variations (CNVs) and Loss of Heterozygosity (LOH) in 31 Brazilian patients with a spectrum of 22q11.2 phenotypic manifestations.

We identified different sizes of genomic alterations in the 22q region. In a cohort of 31 patients (32% males and 68% females) we found 14 deletions and 14 duplications, and 7 patients with loss of heterozygosity including genes associated with lupus erythematosus, polyarthritis, psoriasis, rheumatoid arthritis, sepsis, bronchitis and pneumonia. Hypoparathyroidism, cardiac abnormalities as well as developmental delay and psychiatric disorders were the other features related to some pathogenic CNV showed in these patients.

Cytogenomic investigation permitted a better chromosome breakpoint definition and the identification of deletion and duplication syndromes in 22q region enabling a biological interpretation of the immunologic abnormalities.

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**P11.004****Partial trisomy 22q12.1 and monosomy 13q12.1 in a child due to maternal t(13;22)(q12.1;q12.1)**

S. Altiner<sup>1</sup>, N. Kutlay<sup>1</sup>, T. Tos<sup>2</sup>, T. Tuncali<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, School of Medicine, Ankara University, Ankara, Turkey;

<sup>2</sup>Department of Medical Genetics, Dr. Sami Ulus Maternity and Child Health Hospital, Ankara, Turkey.

Introduction: 22q11.2 microduplication is a well-defined syndrome and its phenotype is variable from mild intellectual disability to severe congenital malformations. Recently, deletion of proximal arm of the 13q is also reported in the literature. Here we report a family with different derivate chromosomes over a 4.5 year-old female patient referred to our department for suspected chromosome breakage syndromes with partial trisomy 22q12.1 and monosomy 13q12.1 originated from maternal t(13;22)(q12.1;q12.1). Material and Methods: Conventional cytogenetics and FISH analyses were performed in all family members using standard methodology. Chromosomal breakage (DEB) test was performed in index case. Karyotypes were then reported according to the ISCN 2013.

Results: Karyotypes of the mother, the index case (DEB test: result) and the male sibling were determined as follows: 46,XX,t(13;22)(q12~13;q11~12), 46,XX,der(22)t(13;22)(q12~13;q11~12) (DEB test: negative) 46,XY,der(13)t(13;22)(q12~13;q11~12).

Conclusion: Index case had the findings of both the partial trisomy of 22q12.1 and the monosomy of 13q12.1 in which severe skeletal malformations, hirsutismus and eventration of diaphragma were due to the latter chromosomal aberration. We suggest a possible role of haploinsufficiency of a gene, FGF9, located at 13q11-12 for the causality.

**P11.005****Clinical and molecular cytogenetic characterization of four unrelated patients carrying 2p14p15 microdeletions**

M. Mathieu<sup>1</sup>, C. Demilly<sup>2</sup>, S. Chantot-Bastaraud<sup>3</sup>, A. Afenjar<sup>3</sup>, C. Mignot<sup>4</sup>, J. Andrieux<sup>5</sup>, M. Gerard<sup>6</sup>, J. Catala-Mora<sup>7</sup>, P. Jouk<sup>8</sup>, A. Labalme<sup>9</sup>, P. Edery<sup>1,9</sup>, D. Sanlaville<sup>1,9</sup>, M. Rossi<sup>1,9</sup>;

<sup>1</sup>Centre de référence des anomalies du développement, Service de génétique, Hôpitaux Civils de Lyon, Lyon, France, <sup>2</sup>Centre de dépistage et prises en charge des troubles psychiatriques d'origine génétique, CH Le Vinatier, Lyon, France, <sup>3</sup>Centre de référence des malformations et maladies congénitales du cervelet, Service de génétique, Hôpital Trousseau, AP-HP, Paris, France, <sup>4</sup>Service de Neuropédiatrie, Hôpital Trousseau, AP-HP, Paris, France, <sup>5</sup>Institut de Génétique Médicale, Hôpital Jeanne de Flandre, CHRU de Lille, Lille, France, <sup>6</sup>Service de génétique, Hôpital Clémenceau, CHU de Caen, Caen, France, <sup>7</sup>Oftalmología Infantil, Hospital el Pilar, Barcelona, Spain, <sup>8</sup>Centre de référence des anomalies du développement, Service de Génétique, Hôpital Couple-Enfant, CHU de Grenoble, Grenoble, France, <sup>9</sup>INSERM U1028 CNRS UMR 5292, UCBL, CRNL, GENDEV Team, Lyon, France.

Chromosomal Microarray (CMA) is a first-tier test in the diagnostic work-up assessment of multiple congenital abnormalities/intellectual disability (MCA/ID) syndromes, detecting pathogenic copy number variations in 15-20% of patients. CMA has allowed the characterization of an increasing number of syndromes, named after their underlying chromosomal unbalance. Even if microdeletions caused by allelic non-homogeneous recombination of repeated duplons in flanking regions generally have a relatively homogeneous size, their clinical phenotype can show variable expressivity, making the prognosis evaluation difficult. It is even more challenging, for clinical geneticists, to evaluate the prognosis of patients carrying "unique" non-recurrent deletions: the information available in the literature is often very poor, mostly concerning only partially overlapping microdeletions. 2p14p15 microdeletions can be considered as a typical example of the latter group of chromosomal abnormalities. Only three patients have been reported to date, showing ID and dysmorphisms. We report the clinical and molecular cytogenetic characterization of four further ID/MCA patients from France and Spain carrying 2p14p15 microdeletions. The present study and a review of the literature showed that 3/7 patients had sensorineural hearing loss. No deafness-causing gene has been reported so far in 2p14. Here we discuss the possible role of the *ACTR2* and *SPRED2* genes in the observed hearing loss. Significant ophthalmological involvement was noted, including glaucoma and rod degeneration. One patient developed a progressive cardiomyopathy, suggesting that a cardiac follow-up should be systematically warranted, even in the absence of congenital heart disease.

In conclusion, our study contributes significantly to delineate the phenotypic spectrum of 2p14p15 microdeletions.

**P11.006****Mild intellectual disability, congenital heart defect and skeletal abnormalities in three patients with 4q13.3 microdeletion**

E. Preikšaitienė, Ž. Maldžienė, B. Aleksiūnienė, V. Kučinskas;

Dept. Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Many patients have genomic alterations that are described in only few individuals and evaluation of clinical significance of these chromosomal alterations is very challenging. Nevertheless detection of novel pathogenic copy number variants and delineation of novel genomic disorders is an important part of genomic studies.

We report on three affected individuals in a family, a mother and her two daughters, 9 and 15 years of age, with mild intellectual disability, minor facial anomalies, congenital heart defect, short stature, broad chest, and scoliosis. The mother and her older daughter additionally had microcephaly and delayed puberty.

Submicroscopic chromosomal alterations were screened by whole-genome genotyping analysis using the HumanCytoSNP-12v2.1 BeadChips (Illumina Inc., San Diego, CA, USA) and revealed 4q13.3 microdeletion, 1.5 Mb in size. FISH analysis using RP11-37J21 probe overlapping part of ADAMTS3 gene confirmed the deletion in all three affected individuals. This novel chromosomal alteration involves GC, NPFFR2, ADAMTS3, COX18 and ANKRD17 genes. ADAMTS3 gene is highly expressed in specific regions of the developing mouse brain and in connective tissues such as bone and tendon.

This clinical report provides clinical and molecular characterization of previously unreported 4q13.3 microdeletion. ADAMTS3 gene might be a candidate gene for intellectual disability and other clinical features, including short stature. Detailed clinical examination of additional patients with a similar microdeletion is needed for further delineation of this clinically recognizable syndrome.

The work was funded by the Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No.CH-3-ŠMM-01/04, UNIGENE project.

**P11.007****Distal 4q partial trisomy vs. distal 10q monosomy - report of a family with a t(4;10)(q31.3;q24)**

G. A. Motei, D. Ozunu, V. Plaiasu;

Mother and Child Care Institute „Prof. Dr. Alfred Rusescu”, Bucharest, Romania.

Distal trisomy 4q and distal monosomy of 10q are rare, complex syndromes of intellectual disability, developmental and behavioral problems and distinctive facial features. The severity of the condition and the signs and symptoms depend on the size and location of the duplication and/or deletion and which genes are involved. Although some cases occur sporadically, most are inherited from a parent with a balanced translocation. Individual cases of trisomy 4q31.3 and deletions of 10q24 are rare and have been previously reported in the literature, with variable clinical manifestations.

We report the case of a boy with partial trisomy for the distal part of the long arm of chromosome 4 (4q31.3-->qter) and a concomitant monosomy 10(10q24-->qter). The patient presented with hydrocephaly, craniofacial dysmorphism, cryptorchidism and axial hypotonia. The chromosomal abnormalities resulted from a paternal balanced translocation involving chromosomes 4 and 10. The inheritance and identity of the translocation was ascertained by extensive familial cytogenetic and FISH studies.

To our knowledge, this report is the first to describe a translocation that resulted in the combination of monosomy 10q24 and trisomy 4q31.3 coexisting in the same individual.

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**P11.008****Mosaic de novo tetrasomy of 5q35 in a newborn with typical features of 5q35 duplication syndrome and heart defect**K. Kuuse, P. Tammaru<sup>1</sup>, P. Ilisson<sup>1</sup>, T. Ilus<sup>1</sup>, M. Jürgenson<sup>1</sup>, O. Žilina<sup>1,2</sup>, K. Muru<sup>1</sup>;<sup>1</sup>Tartu University Hospital, United Laboratories, Department of Genetics, Tartu, Estonia,<sup>2</sup>Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Recently described 5q35 microduplication reciprocal to the common Sotos syndrome deletion (~2 Mb) is associated with microcephaly, short stature, developmental delay and delayed bone maturation. The clinical picture is largely opposite to that of Sotos syndrome (macrocephaly, overgrowth and advanced bone age). The dosage effect of NSD1 (5q35) is suggested to be the cause of the main clinical problems.

We report a dysmorphic newborn with IUGR, microcephaly, short stature,

and prenatally diagnosed congenital heart defect (ASD, VSD, left ventricle hypoplasia). First routine karyotype analysis (GTG-banding) revealed a normal male karyotype. Chromosomal microarray analysis (CMA, HumanCytoSNP-12 BeadChip, Illumina Inc.) revealed a ~8.3 Mb duplication of 5q35.1-qter. To localize the extra copy, FISH analysis was done with Kreatech hTERT/NSD1 and 5p/5q subtelomeric probes. Surprisingly, two additional signals of NSD1 and 5qter were seen, located on a small additional marker chromosome, which was present in ~10% of metaphases (all of them were of bad band quality and so escaped in first analysis), and in 50% of interphase nuclei.

The mosaic tetrasomy due to the presence of der(5q) marker-chromosome in ~50% of cells results in a typical duplication picture seen in CMA as well as in clinical features typical for 5q35 duplication. More severe clinical picture compared to common 5q35 duplication can be explained by encompassing NK2 (cardiac development) and MSX2 (limb and bone development) genes.

**P11.009****Molecular mechanisms of reciprocal rearrangements at 7q11.23**R. Corominas<sup>1,2,3</sup>, G. Palacios-Verdú<sup>1,2,3</sup>, N. Rivera-Brugués<sup>1</sup>, R. Flores<sup>1,2,3</sup>, D. Pérez-García<sup>1,2,3</sup>, G. Aznar<sup>4</sup>, I. Cuscó<sup>1,2,3</sup>, C. A. Morris<sup>5</sup>, C. B. Mervis<sup>6</sup>, L. R. Osborne<sup>7</sup>, L. A. Pérez-Jurado<sup>1,2,3</sup>;

<sup>1</sup>Genetics Unit, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain, <sup>4</sup>Pediatric Neurology, Hospital del Mar, Barcelona, Spain, Barcelona, Spain, <sup>5</sup>Department of Pediatrics, University of Nevada School of Medicine, Las Vegas, NV, United States, <sup>6</sup>Department of Psychological and Brain Sciences, University of Louisville, Louisville, KY, United States, <sup>7</sup>Departments of Medicine and Molecular Genetics and Institute of Medical Science, University of Toronto, Toronto, ON, Canada.

**Introduction**

Williams-Beuren (WBS) and 7q11.23 microduplication (7DUP) syndromes are two neurodevelopmental disorders caused by reciprocal rearrangements at 7q11.23, either deletions (WBS) or duplications (7DUP). These recurrent rearrangements are generated by non-allelic homologous recombination (NAHR) between flanking segmental duplications (SDs).

**Methods**

By dosage analyses of site-specific nucleotides or cis-morphisms, single and multiple-copy microsatellites in trios, we have investigated the extension and parental origin of the rearrangements in 720 WBS and 99 7DUP patients and evaluated the presence of susceptibility factors in transmitting parents. By haplotype cloning and RT-PCR, we have characterized the junction fragments at sequence level in a subset.

**Results**

Deletion events were all de novo, while duplications were mostly de novo (88%) and also inherited (12%). The type and size of the rearrangement was similarly distributed in del/dup cases: 1.55Mb (86%/89%), 1.83Mb (12%/7.5%) and atypical (2%/3.5%). No differences were observed in the parental origin. Disrupted alleles were mediated by inter-chromosomal NAHR in 7q11.23 paracentric inversion carriers in 20%/30% (del/dup) of cases. Rearranged chromosomes resulted in different copy number of the NCF1 gene, and the generation of chimeric GTF2IRD2 transcripts in a subset of probands. We further defined a 1.4Kb hotspot for intrachromosomal NAHR of paternal origin within intron 19 of GTF2I and characterized two additional hotspots for reciprocal inversion-mediated events at intron 2 of GTF2IRD2.

**Conclusions**

Rearrangements causing WBS and 7DUP are the result of reciprocal NAHR events between SDs at 7q11.23. A few hotspots for positional preference of recombination can be identified within SDs.

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**P11.010****9p13.3 Interstitial deletion - a new syndrome?**S. I. Ferreira<sup>1</sup>, I. M. Carreira<sup>1,2,3</sup>, M. Pinto<sup>1</sup>, L. Ramos<sup>4</sup>, J. B. Melo<sup>1,2,3</sup>;

<sup>1</sup>Laboratório de Citogenética e Genómica, Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal, <sup>2</sup>CIMAGO – Centro de Investigação em Meio Ambiente, Genética e Oncobiologia, Coimbra, Portugal, <sup>3</sup>CNC-IBILI, Universidade de Coimbra, Coimbra, Portugal, <sup>4</sup>Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

The 9p deletion syndrome is a clinically characterized syndrome, presenting as a cytogenetically heterogeneous condition with variable deletion sizes. In contrast, 9p interstitial deletions have only been described in five patients. Two patients were characterized by conventional cytogenetics and have larger deletions involving bands 9p12p13, and the other three were

characterized by array-Comparative Genomic Hybridization and have only band 9p13 deleted.

We report a 12 year old male presenting with developmental delay, attention deficit hyperactivity disorder, social personality, short stature, hands tremor, bruxism and facial dysmorphisms that was studied by Agilent 180K oligonucleotide array-CGH. Array-CGH revealed a 2.8Mb de novo interstitial deletion at chromosome 9p21.1p13.3(33,100,287-35,911,318 bp)[hg19], overlapping the previously described patients.

The reported patient presents the smallest 9p13 interstitial deletion as it only includes sub-band 9p13.3, besides 100Kb from band 9p21.1, while the previous described patients have larger deletions including band 9p13. The patients share a 2.1Mb commonly deleted region that includes 65 genes, 34 described in OMIM and 11 in Morbid Map. One of those genes is NPR2, with heterozygous mutations associated with short stature without a distinct clinical phenotype, suggesting that haploinsufficiency of the gene contributes to short stature, a feature reported in the four patients characterized by array-CGH.

These patients share a particular association of clinical features such as developmental delay, attention deficit hyperactivity disorder, social personality, short stature, hands tremor, bruxism and facial dysmorphisms that suggest that 9p13.3 deletion might be a new syndrome.

#### P11.011

##### Balanced X-autosome translocation suggests association between AMMECR1 and growth, bone and heart alterations

**M. Moyses-Oliveira**<sup>1,2</sup>, R. J. Fish<sup>3</sup>, G. Giannuzzi<sup>2</sup>, V. A. Meloni<sup>1</sup>, C. O. Gigek<sup>1</sup>, J. M. Cerutti<sup>1</sup>, A. Di Battista<sup>1</sup>, T. Liehr<sup>4</sup>, N. Kozyakova<sup>4</sup>, G. Carvalheira<sup>1</sup>, M. Neerman-Arbezi<sup>3</sup>, M. I. Melaragno<sup>1</sup>, A. Reymond<sup>2</sup>;

<sup>1</sup>Genetics Division, Department of Morphology and Genetics, Universidade Federal de São Paulo, São Paulo, Brazil, <sup>2</sup>Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland, <sup>3</sup>Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland, <sup>4</sup>Institute of Human Genetics, Jena University Hospital, Friedrich Schiller University, Jena, Germany.

Female balanced X-autosome translocations are usually associated with absence of functional copies of the gene mapping at the breakpoint through disruption of the derivative-chromosome copy and inactivation of the normal X-chromosome. We report on a nine year-old girl with karyotype 46,X,t(X;9)(q23;q12)dn, disproportionate short stature (<1st centile), septal atrial defect, scoliosis, bone dysplasia, and normal cognition. Array-CGH and breakpoint sequencing confirmed the full complement of genetic material, whereas replication banding showed preferential inactivation of the normal X-chromosome. The autosomal breakpoint affects a heterochromatic region while the X-chromosomal breakpoint was mapped between the AMMECR1 and RGAG1 genes. Whereas expression of the latter was unmodified, RT-qPCR indicated absence of AMMECR1 expression in the blood and lymphoblastoid cells of the patient. The encoded AMMECR1 protein, which is evolutionary conserved in prokaryotes and eukaryotes including bacteria, archaea, yeast and mammals, localizes to the nucleus in HeLa cells, and contains nucleic acid-binding RAGNYA domains. We show that AMMECR1 is co-expressed with genes implicated in cell cycle and translation regulation; five of which were previously associated with growth and bone alteration syndromes. Our knockdown of the zebrafish orthologous gene resulted in animals with shorter tails, thin and curved bodies, dorsally-kinked tail-ends, poorly defined somites, pericardial edema and hydrocephaly phenotypes reminiscent of our patient's features. Our results suggest that AMMECR1 is potentially involved in cell cycle control and linked to a new syndrome with growth, bone and heart alterations.

#### P11.012

##### First familial case of Angelman syndrome in Bulgaria

**B. Karashova**<sup>1</sup>, D. Avdjieva-Tzavella<sup>2</sup>, T. Todorov<sup>3</sup>, A. Todorova<sup>4,5</sup>, H. Kathom<sup>6</sup>, S. Tsaneva<sup>7</sup>, I. Litvinenko<sup>8</sup>, R. Tincheva<sup>9</sup>;

<sup>1</sup>University Pediatric Hospital Sofia; Mediacial univestity Sofia, Sofia, Bulgaria, <sup>2</sup>University Pediatric Hospital; Mediacial univestity, Sofia, Bulgaria, <sup>3</sup>Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria, <sup>4</sup>Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria, <sup>5</sup>Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, <sup>6</sup>Department of Clinical Genetics, University Pediatric Hospital, Medical University, Sofia, Bulgaria, <sup>7</sup>Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria, <sup>8</sup>Department of Child Neurology, University Pediatric Hospital, Medical University, Sofia, Bulgaria.

**Introduction:** Angelman syndrome (AS) is a neurodevelopmental disorder caused by genetic defects leading to loss of expression of the maternal copy of the chromosome 15q11-13 imprinted region. Most cases are sporadic, being caused by de novo deletion of maternal chromosome 15q11-13 or by paternal uniparental disomy. Familial cases can occur, due to mutations in the UBE3A gene or in the imprinting center.

**Materials and Methods:** We describe two sibs with mental retardation, absence of speech, and typical facial dysmorphism. The family members were screened by MS-MLPA probemix ME028-B2 Prader-Willi/Angelman for copy number variations and assessment of methylation profile. Both children MS-MLPA profiles were compared with their parents and 3 normal controls.

**Results:** The test for methylation revealed missing SNRPN methylation specific fragments in the patients profile in comparison to their parents and normal controls, which is compatible with paternal non-methylated fragments and deleted or wrong methylated maternal fragments. The copy number test did not show deletion of the SNRPN specific fragments, but two other fragments, corresponding to AS-SRO region (part of the PWS-AS imprinting center) were deleted. The deletion was detected in both affected children and it was inherited from the asymptomatic mother.

**Conclusion:** The missing SNRPN methylation specific fragments correspond to wrong methylation (demethylation) on the maternal allele due to deletion of the PWS-AS imprinting center. This mistake in the methylation profile in both affected children affects maternal copy, while in the asymptomatic mother most probably the deletion affects the paternal copy, hence non-pathogenic.

#### P11.013

##### A de novo Ser to Thr change of a conserved phosphorylation site in aquaporin-4 was found in a man with ID, short stature, deafness and progressive spastic gait disturbance

**S. Berland, G. Houge, III;**  
Haukeland University Hospital, Bergen, Norway.

Aquaporin-4, encoded by AQP4, is the predominant water channel in the brain with importance for the blood-brain barrier, water and electrolyte balance, and edema formation and clearance.

In an adult with mild/moderate ID, sensorineural hearing loss, ataxia, tremor and progressive spasticity, a de novo missense variant was found in AQP4 (NM\_001650.4) c.332G>C, p.(Ser111Thr). Pregnancy and birth had been normal, but shortly thereafter he developed heart failure associated with a cardiomyopathy that later resolved. Neonatal EEG and CT indicated brain ischemic injury, but that also resolved. He had delayed motor and speech development but normal social skills and behavior. A recent cerebral MRI showed large ventricles and atrophic changes in the lentiform nucleus. He is short (L 8 cm < 2.5 p) with a large head (HC 60 cm) and mild facial dysmorphism. Even though no other similar cases have been found, his clinical picture is judged to be compatible with an aquaporin dysfunction.

The S111T change affects a highly conserved phosphorylation site in a short cytoplasmic loop between transmembrane domain 2 and 3 of AQP4, found necessary for channel activation but not regulation of water permeability. Possibly, the change from a serine to a threonine phosphorylation site affects phosphorylation dynamics in a way that disturbs channel fine-regulation upon e.g. local changes in osmolality. This remains to be proven.

#### P11.014

##### Clinical relevance of the BP1-BP2 microdeletion of the chromosome 15 in a patient with epilepsy and developmental delay

**A. Szabó**<sup>1,2</sup>, M. Czakó<sup>1,2</sup>, K. Hadzsiev<sup>1,2</sup>, A. Lőcsei-Fekete<sup>1,2</sup>, B. Duga<sup>1,2</sup>, K. Komlósi<sup>1,2</sup>, E. Pöstyéni<sup>1,2</sup>, T. Szabó<sup>1,2</sup>, B. Melegi<sup>1,2</sup>;

<sup>1</sup>Department of Medical Genetics, Pécs, Hungary, <sup>2</sup>Szentagothai Research Centre, Pécs, Hungary.

In the 15q11.2-q13 region five recurrent breakpoints (BP1 to BP5) have been observed. The most common rearrangements are the type I (BP1-BP3) and II (BP2-BP3) deletions associated with well known phenotype (Prader-Willi and Angelman syndromes). Clinical manifestations of type I and II deletions include speech and motor developmental delay, behavioral and characteristic dysmorphic features, however, BP4-BP5 microdeletions comprising CHRNA7 lead to intellectual disability accompanied by epilepsy. Furthermore, in a couple of cases with less severe symptoms, BP1-BP2 (15q11.2) deletions have been published. We investigated a boy having developmental delay, epilepsy and muscle hypotonia using Agilent Human Genome Unrestricted G3 ISCA v2 Sureprint 8x60K oligo-array. Array-CGH revealed a 0.4 Mb deletion of the 15q11.2 BP1-BP2 region which contains only four highly conserved genes, TUBGCP5, NIPA1, NIPA2 and CYFIP1. The association of the concerned genes with the phenotype of our patient and of a few known cases has been suggested earlier. Based on our results we can confirm that haploinsufficiency of the TUBGCP5, NIPA1, NIPA2 and CYFIP1 genes could lead to epilepsy, developmental delay, generalized hypotonia and dysmorphic features. Despite the small number of the affected genes, it is very difficult to set up a correct clinical diagnosis in patients with BP1-

BP2 deletions due to the non-specificity of the symptoms, which emphasize the application of the array-CGH in cases with similar phenotype. This research was supported by Hungarian Scientific Research Fund: 103983.

#### P11.015

#### Severe arterial tortuosity syndrome due to a rare *SLC2A10* gene mutation

*M. Kocova<sup>1</sup>, K. Kuzevska-Maneva<sup>1</sup>, R. Kacarska<sup>1</sup>, V. Anastasovska<sup>1</sup>, E. Sukarova-Angelovska<sup>1</sup>, M. Ritelli<sup>2</sup>, M. Colombi<sup>2</sup>*

<sup>1</sup>Pediatric Clinic, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>Department of Molecular and Translational Medicine, School of Medicine, University of Brescia, Brescia, Italy.

Arterial tortuosity syndrome (ATS) is a very rare autosomal recessive disorder mainly characterized by tortuosity and elongation of the arteries and by dyspnea, cyanosis, dysmorphic features, joint hypermobility and skeletal anomalies. Infancy and childhood are the most critical for life-threatening events, such as acute respiratory symptoms, due mostly to complications of stenosis of the pulmonary arteries. Vascular changes might cause aneurysms or artery dissections later in life. The causal gene *SLC2A10* encodes the facilitative glucose transporter 10 and nearly 100 patients and 23 mutations are known.

We report a 10-year-old Macedonian girl who was referred due to easy fatigue and visible vascular pulsations on the neck. She had recurrent dyspnea and moderate cyanosis with effort, but no heart defect was diagnosed in early childhood. She was a slender girl with joint hypermobility, narrow face, high-arched palate and crowded teeth. Enlarged, elongated, and extremely tortuous large and medium size blood vessels were detected on ultrasonography and magnetic resonance angiography. *SLC2A10* molecular analysis revealed homozygous c.254T>C(p.Leu85Pro) mutation in the patient. Her parents were unrelated heterozygous carriers. Interestingly, the same mutation was previously found in two Macedonian brothers with a severe clinical presentation. Detailed family history evaluation revealed that the present patient is a second cousin of these two brothers, with parents being first cousins. However, no consanguinity with the partners was reported.

In conclusion, we describe a patient/family with a mutation causing severe ATS. Molecular analysis is important for genetic counseling and to define targeted clinical follow-up and timely cardiovascular surgery when needed.

#### P11.016

#### A recurrent translocation t(1;12)(q43;q21.1) drawn from a systematic survey of balanced translocations in Finns

*T. M. Luukkainen<sup>1,2</sup>, M. Pöyhönen<sup>3,4</sup>, M. M. Mehrjouy<sup>5</sup>, A. Palotie<sup>1,6</sup>, A. Anttonen<sup>7</sup>, T. Varilo<sup>2,3</sup>*

<sup>1</sup>Institute for Molecular Medicine Finland FIMM, Helsinki, Finland, <sup>2</sup>Dept. of Health, National Institute for Health and Welfare, Helsinki, Finland, <sup>3</sup>Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>4</sup>Dept. of Medical Genetics, University of Helsinki, Helsinki, Finland, <sup>5</sup>Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark, <sup>6</sup>Broad Institute of Harvard and MIT, Cambridge, MA, United States, <sup>7</sup>Laboratory of Genetics, HUSLAB, Helsinki, Finland.

Reciprocal translocations are the most frequent human chromosomal aberrations. At least 6-9% of de novo balanced translocations are associated with abnormal clinical phenotypes. As an outcome of the Systematic Survey of Balanced Chromosomal Rearrangements in Finns, we identified two unrelated families with balanced t(1;12)(q43;q21.1).

In collaboration with the 'International Breakpoint Mapping Consortium IBMC', the breakpoint regions were narrowed down to ~1.3 kb and ~1.25 kb intervals, respectively, using mate-pair sequencing. Sanger sequencing in one family mapped the exact breakpoints to 239,567,377-239,567,379 on chromosome 1 and 73,989,462-73,989,463 on chromosome 12. Analysis of the flanking sequences revealed a breakpoint intersecting Alu element on chromosome 1, and another Alu element in close vicinity of the breakpoint on chromosome 12.

In the first family, carriers (n=6) manifest first with learning problems and later with neurological symptoms (chronic headache, balance problems, tremor, fatigue), episodic ataxia type III, and cerebral infarctions without identified predisposing factor. The carriers in the second family (n=4) demonstrate a variable phenotype including tetralogy of Fallot, transient ischaemic attack, valvular regurgitation, arrhythmia, migraine or hypertension. In both families the translocation seems to co-segregate with the abnormal phenotype. No protein coding genes are affected by the breakpoints, however, we identified *CHRM3* (cholinergic receptor, muscarinic) on chr1, and *KCNC2* (voltage gated potassium channel) and *ATXN7L3B* (ataxin 7-like 3B) on chr12 closest to the translocation breakpoints.

This study demonstrates how a recurrent translocation t(1;12)(q43;q21.1) can result in different phenotypes involving both vascular and neurological manifestations.

#### P11.017

#### Case report: A novel mutation in Bardet-Biedl syndrome 8

*M. Mirjalili<sup>1,2</sup>, F. Harrazi<sup>1</sup>, S. Seyedhassani<sup>1</sup>*

<sup>1</sup>Dr.Seyedhassani medical genetic center, Yazd, Iran, Islamic Republic of, <sup>2</sup>Department of Biology, Faculty of Sciences, Yazd University, Yazd, Iran, Islamic Republic of.

Introduction: Bardet-Biedl syndrome (BBS) is an autosomal recessive multi-systemic human genetic disorder characterized by six major defects including obesity, mental retardation, renal anomalies, polydactyly, retinal degeneration and hypogenitalism. In several cases of BBS, few other features such as metabolic defects, cardiovascular anomalies, speech deficits, hearing loss, hypertension, hepatic defects and high incidence of diabetes mellitus have been reported. The BBS displays extensive genetic heterogeneity. To date, 19 genes have been defined as related to BBS.

In this study, we analyzed 18 BBS-related genes in a patient by NGS technique.

Materials and methods: Panel-based next generation sequencing as a reliable technique to detect mutations in 18 BBS-associated genes was used.

Results: NGS detected one novel mutation c.936delA in coding exon 11 of *TTC8* gene that related to BBS type 8. Bioinformatic analysis (CADD score:22.6) confirmed that this mutation is pathogenic in nature. This deletion creates a frame shift that leads to early termination of aminoacid coding, which is expected to affect the function of protein.

Conclusions: We found a novel mutation in *TTC8* gene that related to BBS type 8. This finding can be useful in genetic counseling and Prenatal diagnosis during next pregnancy.

#### P11.018

#### Homozygous truncating *CEP19* mutation causing novel Bardet-Biedl syndrome

*E. Yıldız<sup>1</sup>, S. Mumtaz<sup>2</sup>, M. Afzal<sup>2</sup>, S. Malik<sup>2</sup>, A. Tolun<sup>1</sup>*

<sup>1</sup>Bogazici University, Istanbul, Turkey, <sup>2</sup>Quaid-i-Azam University, Islamabad, Pakistan.

Introduction: Bardet-Biedl syndrome (BBS) is a rare, pleotropic ciliopathy with highly variable phenotype. Primary features are retinal dystrophy, obesity, post-axial polydactyly, urino-genital abnormalities and intellectual disabilities. We investigated a consanguineous BBS Pakistani family afflicted with a primary presentation of polydactyly and obesity and accompanying intellectual disability, diabetes and behavioral problems.

Materials and Methods: The disease locus was identified by linkage mapping using SNP genotype data of 14 family members. Whole exome sequencing for two affected subjects revealed rare/novel variants. Results: At the disease locus, an approximately 4.5-Mb region at 3q29 yielding a multipoint LOD score of 3.69, homozygous nonsense *CEP19* c.194\_195insA (p.Tyr65\*) mutation was identified, for which all the affected individuals plus an unaffected sibling were homozygous. Some affected members of the study family also carry the rare *CCDC28B* variant reported as a modifier of BBS phenotype

Conclusion: *CEP19* is a novel gene for BBS, with reduced penetrance. It encodes a coiled coil domain containing ciliary protein that localizes to the primary cilia as do some other BBS proteins. The protein sequence is totally conserved between human and chimpanzee. The gene is expressed in several organs. It has been associated with obesity with azospermia in a family. Although the low frequency of the *CCDC28B* variant indicates that the presence of the variant is not circumstantial, the variant did not correlate with phenotype severity. Absence of any renal disturbance in the family can be attributed to the absence of expression of the gene in kidney.

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#### P11.019

#### A new case of monozygotic male twins discordant for Beckwith-Wiedemann syndrome caused by KvDMR1 hypomethylation

*M. J. Sánchez-Soler<sup>1</sup>, M. C. Martínez-Romero<sup>2</sup>, V. López-González<sup>1</sup>, M. J. Ballesta-Martínez<sup>1</sup>, J. Susmozas-Sánchez<sup>3</sup>, S. E. Boonen<sup>4</sup>, A. B. Skytte<sup>4</sup>, G. Glóver<sup>2</sup>, E. Guillén-Navarro<sup>5</sup>*

<sup>1</sup>Medical Genetic Sección, H. Clinic Universitary V. Arrixaca, IMIB-Arrixaca, El Palmar, Spain, <sup>2</sup>Centro de Bioquímica y Genética Clínica, H. Clínico Universitario V. Arrixaca, IMIB-Arrixaca, El Palmar, Spain, <sup>3</sup>Servicio de Neonatología, H. Clínico Universitario V. Arrixaca, El Palmar, Spain, <sup>4</sup>Department of Clinical Genetics Aarhus University Hospital, Aarhus, Denmark, <sup>5</sup>Consejería de Sanidad, Murcia, Spain.

In most cases monozygotic twins (MT) are concordant for genetic diseases,

but more than 15 cases of MT discordant for Beckwith-Wiedemann syndrome (BWS) have been described. Incidence of MT among BWS patients is higher than among general population (2.5-8% vs 0.3-0.4%), but it is unclear whether the imprinting defect may trigger the twinning process, or it is the other way around.

We report a case of male MT BWS discordant, naturally conceived without any significant family history. Mother presented gestational diabetes controlled by diet, and in 24 weeks ultrasound, severe hydrocephaly and polyhydramnios was detected in the 2nd twin. Delivery was at 26+6 weeks, and both were admitted to NICU. 1st twin (BWS) weighed 1000g (61th percentile). He developed bronchopulmonary dysplasia (BDP) and apneas consequence of macroglossia, without other complications. He had capillary malformation on the forehead, coarse features, inguinal hernia, but neither hemihypertrophy nor psychomotor delay. Methylation study performed in blood and buccal swab showed KvDMR1 hypomethylation. 2nd twin weighed 1050g. He underwent surgery in the first month because of hydrocephaly consequence of aqueduct stenosis. He also developed BPD, and mild psychomotor limitations secondary to central nervous system infection following the operation. Methylation study was normal in both samples. This is the first case in which KvDMR1 hypomethylation discordance has been detected in blood sample, suggesting that the imprinting defect occurred after the twinning process, maybe due to an inadequate maintenance of methylation state. Specific environmental factors or ART use were not documented, so other predisposing factors should be implicated.

#### P11.020

#### Unraveling unusual presentations of classical syndromes by exome sequencing: the case of primary microcephaly

A. Verloes<sup>1</sup>, S. Passemard<sup>2</sup>, M. Schiff<sup>3</sup>, Y. VIAL<sup>2</sup>, C. Dupont<sup>4</sup>, M. Ouachée<sup>4</sup>, J. Viala<sup>5</sup>, E. Cuadro<sup>6</sup>, R. Kom<sup>7</sup>, S. Drunat<sup>2</sup>;

<sup>1</sup>Clinical Genetic Dept, PARIS, France, <sup>2</sup>Clinical Genetic Dept, Robert DEBRE Hospital, PARIS, France, <sup>3</sup>Child Neurology Dept, Robert DEBRE hospital, PARIS, France, <sup>4</sup>Clinical Hematology Dept, Robert DEBRE Hospital, PARIS, France, <sup>5</sup>Child Gastroenterology Dept, Robert DEBRE Hospital, PARIS, France, <sup>6</sup>Dept of Pediatrics, Cayenne Hospital, Cayenne (French Guiana), France.

We illustrate situations where exome sequencing allowed „salvage“ of diagnoses missed because the typical clinical picture is absent. The first case is a 10 years girl, from Guadeloupe island, with IUGR, congenital microcephaly, gastrostomy, and late onset susceptibility to infections. She had multiple CAL spots, but no skin photosensitivity. Biallelic BLM mutations were found by exome, and the diagnosis of Bloom syndrome, was confirmed by SCE study. We missed it because the facial pseudo-lupic rash was lacking, and disorality was of unusual severity. The second patient, a 4 years girl, had severe congenital microcephaly, progressive spasticity, profound ID, and no Ichthyosis. Her EEG showed a disorganized pattern, but no epileptic discharges. She never presented seizures. Her similarly affected elder brother, died at 4 months of gastroenteritis. Seizures were not reported. In both, MRI showed small brain without malformation. Conventional blood screenings (including AA chromatography) showed no abnormalities. CSF was not investigated in the absence of epilepsy. By exome, we found biallelic mutations in PHGDH, coding the first enzyme in the serine synthesis pathway. Very low serine level in CSF confirmed the diagnosis. Microcephaly, deep delay and spasticity are typical of serine synthesis defects, but the very early appearance of refractory epilepsy is considered as constant. We illustrate how „simple“ diagnosis can be missed when missing key sign misplaces diagnostic thinkings. We also illustrate the danger of restricting access to targeted diagnostic DNA panels. The generalization of diagnostic exomes will certainly lead to expand the clinical spectrum of known Mendelian entities.

#### P11.021

#### PLS3-related bone disorder in brothers with syndromic features

E. Nishi<sup>1,2</sup>, T. Kosho<sup>1,2</sup>, K. Masuda<sup>3</sup>, Y. Kato<sup>3</sup>, K. Shirahige<sup>3,4</sup>, K. Izumi<sup>1,3</sup>;

<sup>1</sup>Division of Medical Genetics, Nagano children's hospital, Azumino city, Japan,

<sup>2</sup>Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan, <sup>3</sup>Research Center for Epigenetic Disease, Institute for Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan, <sup>4</sup>CREST, Japan Science and Technology Agency, Kawaguchi, Austria.

Plastin 3 (PLS3) is a protein involved in the formation of filamentous actin bundles. Recently, a total of 21 individuals from 8 families have been reported with PLS3-related familial osteoporosis/osteoporotic fractures (van Dijk et al., 2013; Fahiminiya et al., 2014; Laine et al., 2015). We report on an additional family with a novel PLS3 variant, identified through genetic screening for syndromic developmental delay.

The proband is a 12-year-old boy manifesting facial characteristics (hypertelorism, upslanted palpebral fissures, broad nasal tip, blue sclerae), mild

developmental delay, hearing impairment, inguinal/umbilical hernia, joint laxity, and recurrent fractures associated with decreased bone mineral density. His younger brother had similar manifestations. SNP array analysis detected no pathogenic copy number variations. Exome sequencing revealed a novel missense variant in PLS3 in both of the brothers, which was detected in their mother. The variant, altering highly conserved amino acid and localized at the CH2 actin binding domain 1 of PLS3 protein, was presumed to be pathogenic through disrupting the interaction between PLS3 and actin. Previously reported patients with PLS3 mutations, loss-of-function type in most, had osteoporosis with/without osteoporotic fractures, and only two were described to have non-skeletal features (epilepsy, spastic cerebral palsy). Manifestations shared by the present brothers implicate a wider phenotypic spectrum of PLS3 abnormalities, though non-skeletal features in both of them might have been caused by other genetic factors.

#### P11.022

#### A case report of 6p25p22 duplication associated with BOFs clinical features

P. Rendeiro<sup>1</sup>, T. Lourenço<sup>2</sup>, R. Lemos<sup>1</sup>, C. Ventura<sup>1</sup>, S. M. Pereira<sup>1</sup>, J. Pinto-Basto<sup>1</sup>, J. Sá<sup>1</sup>, P. Tavares<sup>1</sup>;

<sup>1</sup>CGC Genetics, Porto, Portugal, <sup>2</sup>CGC Genetics, Lisboa, Portugal.

Branchio Oculo Facial syndrome (BOFs\_MIM:113620) is a rare autosomal dominant disorder caused by haploinsufficiency of the transcription factor AP-2 alpha gene (TFAP2A). TFAP2A is known to activate gene transcription crucial to embryo development, in particular, embryogenesis of the eye, ear, face, body wall, limbs and neural tube. Three major clinical indications may assist a clinical diagnostic of BOFs: branchial cleft sinus defects, ocular anomalies, and characteristic facial anomalies, such as cleft or pseudo cleft lip and palate. Nevertheless, this disorder has been characterized with variable expression between patients. The genetic makeup of individuals with BOF syndrome always compromises TFAP2A function and normally is presented in the form of a microdeletion, a complex deletion/de novo insertion or point mutation(s) within TFAP2A. We report a 2yo boy with classic clinical features of BOF syndrome and an interstitial duplication on 6p25.2p22.3. To the best of our knowledge, so far no patient with BOF syndrome has been reported to carry a duplication on 6p25.2p22.3.

Array CGH was performed on an Affymetrix platform, Cytoscan 750K. Data analysis was performed on ChAS Software, Affymetrix (reference NCBI\_hg19). Karyotype was performed on peripheral blood following standard protocols.

Patient's examination demonstrated clinical manifestations typical of BOF syndrome, including branchial, ocular and facial anomalies, such as right cervical branchial cyst, blepharophimosis and dysmorphic features. Array CGH revealed a 13.9Mb duplication at 6p25.2p22.3 (Chr6:4,066,234 18,025,300), resulting in a complete duplication of TFAP2A and 75 other genes. Karyotype analysis allowed definition of the type of structural rearrangement present in the patient [46,XY,dup(6)(p25.2p22.3)].

#### P11.023

#### Cat eye syndrome often without cat eye?

G. Cardos<sup>1</sup>, R. Costache<sup>2</sup>, L. Oprea<sup>1</sup>, O. Mantescu<sup>1</sup>, M. Malageanu<sup>1</sup>, S. Grigore<sup>1</sup>, D. Craiu<sup>3</sup>, V. Radoi<sup>1,2</sup>;

<sup>1</sup>Synevo Central Laboratory, Bucharest, Romania, <sup>2</sup>Genetics Division, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, <sup>3</sup>Pediatrics Neurology Division, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Cat eye syndrome is a rare condition involving a partial trisomy or tetrasomy of part of chromosome 22, clinically characterized by highly variable congenital malformations. The syndrome name is derived from a particular appearance of the vertical colobomas ("cat eye") in the eyes of several patients. We present here a case of a patient diagnosed with cat eye syndrome, without "cat eye".

A two-year-girl was ascertained through non-specific developmental delay, hypotonia, major anomalies (including anal atresia, spontaneously resolving ASD, possible Pierre Robin sequence characterized by

microretrognathia and bifid uvula), plus minor craniofacial dysmorphisms - including pre-auricular tags. It inspired an interesting and captivating differential diagnosis to the genetics team, considering disorders not limited to: a possible cat eye syndrome, duplication 22q11.2 syndrome, Emmanuel syndrome, or rather a variant of unbalanced translocation involving a chromosome 22 and another cytogenetic partner.

Classical cytogenetics analysis revealed an acrocentric derived supernumerary marker chromosome (GTG banding), satellited at one end (Ag NOR banding), and made up of a lot - but not exclusively - heterochro-

matic

material (CBG banding). Spectral karyotyping identified its origin in chromosome 22 and chromosomal microarray showed a 3 MB partial tetrasomy 22q11.1-22q11.21 encompassing the cat eye critical region.

The above mentioned chromosomal conditions originating in 22q abnormalities have an overlapping spectrum and sometimes they are a difficult call for the geneticist; further cytogenetic investigations are protean in such cases. Cat eye syndrome without cat eye (coloboma) may be more frequent than we have thought.

## P11.024

### CDC42 as a new human disease gene associated with thrombocytopenia and intellectual disability

**K. Kosaki<sup>1</sup>, T. Takenouchi<sup>1</sup>, T. Uehara<sup>1</sup>, S. Iida<sup>2</sup>, R. Kosaki<sup>3</sup>, N. Okamoto<sup>4</sup>;**

<sup>1</sup>Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan,

<sup>2</sup>Department of Gastroenterology and Endocrinology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, <sup>3</sup>Division of Medical Genetics, National Center for Child Health and Development, Tokyo, Japan, <sup>4</sup>Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan.

CDC42 encodes critical regulator for the cell cycle and actin cytoskeleton formation. More than 5000 articles have been published concerning Cdc42 in various organisms. The conditional homozygous knockout of Cdc42 in mice results in macro-thrombocytopenia and structural abnormality in the central nervous system. However, no human disorder associated with CDC42 mutations has been described until our identification of two patients with de novo CDC42 (p.Tyr64Cys) mutation [MIM 616737: Takenouchi-Kosaki syndrome]. The two patients shared macro-thrombocytopenia, developmental delay, lymphedema of the lower extremities, and contracture of the fingers as common features. Characteristic facial features included arched eyebrows, mild ptosis, eversion of the lateral portion of the lower eyelid, exotropia, midfacial hypoplasia, short philtrum, thin upper lip, and malocclusion. The present observation of the two unrelated patients with a strikingly similar phenotype and the same CDC42 mutation establishes that CDC42 is a new human disease gene and that a mutation in CDC42 causes a recognizable syndromic form of thrombocytopenia. Differential diagnosis of isolated thrombocytopenia and intellectual disability include Jacobsen syndrome (11q23 deletion), Braddock-Carey syndrome (21q22), and Takenouchi-Kosaki syndrome. Takenouchi-Kosaki syndrome caused by CDC42 mutation is clinically recognizable in that the size of the platelets is large and lymphedema is a unique feature. Lymphedema could be ascribed to the CDC42 mutation, since Cdc42 directly interacts with Rac1, the defect of which leads to lymphedema in mice.

## P11.025

### Molecular characterization of the first Spanish case of Hypotrichosis with juvenile macular dystrophy (HJMD)

**F. Blanco-Kelly, L. Rodriguez da Silva-Jacy, I. Sanchez-Navarro, R. Riveiro-Alvarez, M. Lopez Martinez, M. Corton, C. Ayuso;**

*Servicio de Genética, ISS-Fundación Jiménez Díaz, Madrid, Spain.*

**Introduction:** The CDH3 gene on 16q2 is responsible for two ultrarare autosomal recessive disorders characterized by hypotrichosis and progressive macular dystrophy: Hypotrichosis with Juvenile Macular Dystrophy (HJMD) and Ectodermal Dysplasia, Ectrodactyly and Macular Dystrophy (EEM).

**Patients and methods:** A Spanish male born in 1998 from non-consanguineous healthy parents sent to the Genetic Department from University Hospital Fundación Jiménez Díaz, with a suspected diagnosis of decalvant spinulous follicular keratosis (probable Siemens Syndrome) and inverse retinitis pigmentosa. Neither limb nor dental abnormalities were observed. Sanger sequencing of coding exons of ABCA4 and CDH3 were performed. In addition, MLPA analysis using a commercial kit was used to study ABCA4 exonic deletions or duplications.

**Results:** First, differential diagnosis of isolated juvenile macular dystrophy was performed. Only a heterozygous missense p.Val2050Leu variant in ABCA4 was found after a comprehensive analysis of coding regions and gene rearrangements. A second allele was not found.

Further CDH3 sequencing allowed to detect compound heterozygous variants: a novel maternal missense change p.Val205Met, predicted as probably pathogenic by *in silico* analysis and a previously reported paternal frameshift c.830del p.Gly277Alafs\*20. Clinical revision allowed reclassified this patient as HJMD.

**Conclusions:** This is the first report of a Spanish patient with HJMD and the first report of a patient carrying mutations in the CDH3 gene in Spain. A new mutation has been described in CDH3. This work reflects the importance of both the joint assessment of clinical signs and the evaluation of the pedigree for a correct genetic study approach and diagnostic.

## P11.026

### The face of the developmental disorders of chromatin remodeling

**S. Douzgou<sup>1,2</sup>, S. Banka<sup>2,1</sup>;**

<sup>1</sup>Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom, <sup>2</sup>Institute of Human Development, University of Manchester, Manchester, United Kingdom.

Developments in technology led to increased understanding of the genetic basis of the Chromatin Disorders, a group of developmental disorders caused by disruption of chromatin remodelling (DDCRs). We used Facial Dysmorphology Analysis technology (FDNA®) to objectively evaluate the craniofacial dysmorphic features of DDCRs. We propose that a condition should be recognizable by gestalt if it fulfills the following criteria: (i) the 'syndromic face' is significantly different from the average, general population face (denoted as 'severity'); (ii) the syndromic face is significantly different from typical faces of other syndromes (denoted as 'distinctiveness'); (iii) the variability between faces of patients with the same syndromic diagnosis is minimal.

As variability is difficult to assess without knowing the full spectrum of the conditions, we focused our analysis on the severity and distinctiveness of DDCRs by using published, 2D facial photographs of patients with 20 distinct, molecularly confirmed DDCRs to create a common 'facial photo crop' for DDCRs. This was proven to be distinct to the 'average, normal face'. To evaluate the severity and distinctiveness of the DDCR faces, we compared each of the 20 syndromes' facial photo crops (n=5) to 1) other syndromes in the DDCR group (19 syndromes); 2) other dysmorphic syndromes not in the DDCR group (100 other syndromes); 3) normal (1000 photographs). Methods included the mean area under the curve (AUC) to compare between samples and ROC curve plotting the true positive rate as function of false positive rates. We report the results of this analysis and discuss its implications.

## P11.027

### Early results of next-gen cytogenetics implementation in Portugal

**D. David<sup>1</sup>, J. Freixo<sup>2</sup>, B. Marques<sup>1</sup>, I. Carvalho<sup>2</sup>, N. Tkachenko<sup>3</sup>, N. Oliva Teles<sup>3,4</sup>, M.**

**Marques<sup>1</sup>, M. Cardoso<sup>1</sup>, J. Fino<sup>1</sup>, A. Alves<sup>1</sup>, A. Fortuna<sup>3,4</sup>, S. Dória<sup>5</sup>, C. Pinto de Moura<sup>5</sup>, H. Correia<sup>1</sup>, I. Marques Carreira<sup>6</sup>, J. Sá<sup>7</sup>, R. Gonçalves<sup>2</sup>, J. Lavinha<sup>1</sup>, T. Kay<sup>2</sup>, M. Talkowski<sup>8,9,10</sup>, C. Morton<sup>9,10,11</sup>;**

<sup>1</sup>National Health Institute "Doutor Ricardo Jorge", Lisbon, Portugal, <sup>2</sup>Centro Hospitalar de Lisboa Central, Lisbon, Portugal, <sup>3</sup>Centro de Genética Médica Doutor Jacinto

Magalhães, Centro Hospitalar do Porto, Porto, Portugal, <sup>4</sup>Unit for Multidisciplinary Research in Biomedicine (UMIB/ICBAS), Porto, Portugal, <sup>5</sup>Faculdade de Medicina, Universidade do Porto, Porto, Portugal, <sup>6</sup>Faculdade de Medicina, Universidade de

Coimbra, Coimbra, Portugal, <sup>7</sup>Centro Hospitalar de Coimbra, Universidade de Coimbra, Coimbra, Portugal, <sup>8</sup>Massachusetts General Hospital, Boston, MA, United States,

<sup>9</sup>Harvard Medical School, Boston, MA, United States, <sup>10</sup>Broad Institute of MIT and Harvard, Cambridge, MA, United States, <sup>11</sup>Brigham and Women's Hospital, Boston, MA, United States.

**Background:** Most approaches are insensitive to the full mutational spectrum of chromosome rearrangements associated with human developmental abnormalities. Therefore, our aim is to introduce next-generation sequencing (NGS) into clinical cytogenetics, creating a sequence-based Next-Gen Cytogenetics to catalyze a dramatic advancement in clinical diagnostics.

**Methods:** Twenty families with chromosome rearrangement-associated diseases, including two prenatal (PN) cases, have been enrolled. Fourteen of these were also analyzed by NGS using large-insert paired-end libraries.

**Results:** The majority of these cases were confirmed to be balanced reciprocal rearrangements, whereas 4 were complex chromosomal rearrangements including 1 of chromothripsis. Thus far, over 50 breakpoints were identified disrupting protein coding genes, lncRNAs, or intergenic regions, thus revealing candidate genes or genomic loci. These cases are further assessed for pathogenicity from positional effects on genes located within topological domains (TADs) containing the breakpoints using DECIPHER predictions of haploinsufficiency. In one PN case, the 16q24 breakpoint disrupts ANKRD11, etiologic in the autosomal dominant KBG syndrome (OMIM #148050), predicting an abnormal phenotype. The chromothripsis case, submitted as 46,XY,t(7;14)(q22;q32.1),inv(15)(q21.2q26.1), proved by NGS to carry two further deletions, at 3p12 (5.3 Mb) and 15q14 (488 kb), as well as an insertion of 644.4 kb from 15q14 into 3p14. The inv(15) is in fact a complex rearrangement of 15q with eight breakpoints.

**Conclusions:** We demonstrate that NGS-based chromosomal rearrangement characterization leads to major improvements in identification of chromosomal aberrations and in prediction of clinical outcomes of postnatally and prenatally detected genomic rearrangements, and to contributions to human genome annotation.

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**P11.028****CLAPO Syndrome: An update on phenotype and molecular approaches**

R. Martín Arenas<sup>1</sup>, V. Martínez-Glez<sup>2</sup>, P. Lapunzina<sup>2</sup>, E. Vallespín<sup>2</sup>, V. Fernández-Montaño<sup>2</sup>, Á. Del Pozo<sup>2</sup>, J. Silla-Castro<sup>2</sup>, K. Ibanez<sup>2</sup>, J. López-Gutiérrez<sup>3</sup>, L. Rodríguez-Laguna<sup>2</sup>;

<sup>1</sup>INGEMM-IdiPAZ, Madrid, Spain, <sup>2</sup>INGEMM-IdiPAZ-CIBERER, Madrid, Spain, <sup>3</sup>Hospital Universitario La Paz, Madrid, Spain.

**Introduction:** CLAPO syndrome (OMIM:613089) is a vascular/overgrowth disorder characterized by Capillary malformation (CM) of the lower lip, Lymphatic malformation (LM) of the face and neck, Asymmetry, and Partial Overgrowth. Six cases have been reported so far. Inheritance pattern is still unknown, but somatic mosaicism is suspected.

**Methods and Results:** We performed a retrospectively review of CLAPO patients diagnosed in our hospital (10 cases) between 2008 and 2015, the largest cohort to date. The hallmark clinical feature is the CM of the lower lip, expressed congenitally in all patients; all other features can be present at birth or develop at a later stage in life. Eight patients showed LMs involving oral mucosa and neck. LM seems to be more frequent in the oral cavity, specifically in tongue, and it is commonly complicated with severe haemorrhagic events. Five patients showed asymmetric overgrowth at birth, and only one presented generalized overgrowth. As a preliminary molecular approach we performed NGS experiments (100x) in paired blood/tissue samples, using a custom panel covering about 300 vascular/overgrowth related genes, including *PIK3CA*, *PTEN*, etc. No germinal/somatic mutations were detected.

**Conclusion:** Here, we present an expansion of the phenotype of CLAPO syndrome, in which we more clearly define some of the originally described clinical manifestations. We have also discarded, at medium reading depths, the presence of somatic mosaic mutations in genes known to cause clinically related syndromes. Our future approach includes increasing reading depth in candidate genes, for better detection of low mosaics, and to perform whole exome NGS.

**P11.029****28 de novo CNVs suggested as candidate regions for CL±P in a high prevalence area in South America**

D. Mattos<sup>1</sup>, F. M. de Carvalho<sup>2</sup>, R. E. Fonseca<sup>1</sup>, F. A. Poletta<sup>3</sup>, A. R. Vieira<sup>4</sup>, P. Lapunzina<sup>5</sup>, E. E. Castilla<sup>2</sup>, I. Orioli<sup>1</sup>;

<sup>1</sup>ECLAMC (Latin American Collaborative Study of Congenital Malformations) and INAGEMP (National Institute of Population Medical Genetics) at Department of Genetics, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil,

<sup>2</sup>ECLAMC (Latin American Collaborative Study of Congenital Malformations) and INAGEMP (National Institute of Population Medical Genetics) at LEMC (Laboratory of Congenital Malformations Epidemiology), Oswaldo Cruz Institute/Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, <sup>3</sup>ECLAMC (Latin American Collaborative Study of Congenital Malformations) at CEMIC (Center for Medical Education and Clinical Research) and CONICET (National Council for Scientific and Technical Investigation), Buenos Aires, Argentina, <sup>4</sup>Departments of Oral Biology and Pediatric Dentistry and Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, United States, <sup>5</sup>Medical and Molecular Genetics Institute (INGEMM), IdiPAZ, La Paz Hospital and CIBER of Rare diseases (CIBERER), Madrid, Spain.

**Background:** A high prevalence of cleft lip with or without palate (CL±P) was detected in Patagonia (Argentina) and Amerindian ancestry appointed as a risk factor. In this presumably more homogenous population, we searched for de novo copy number variation (CNV) in CL±P subjects.

**Method:** 30 families with isolated CL±P (31 affected and 113 total individuals) were genotyped on the Affymetrix Genome-Wide SNP 6.0 array. CNVs identification was performed on Genotyping Console and CHAS (Affymetrix). From all identified CNVs from affected subjects, we filtered out those with lesser chances of pathogenicity according to the following criteria, considering a minimum of 60% overlap: (1) mean-maker-distance higher than 5kbp; (2) CNVs also present in healthy relatives; (3) common CNV from DGV and (4) within 1mbp from telomeres and centromeres.

**Results:** We detected 28 de novo CNVs larger than 200kbp, 6 of which overlapped genes previously associated with CL±P. Five were deletions (no homogenous loss) and 23 duplications. Five segments were detected in 3 subjects and 7 were detected in 2 subjects, indicating recurring de novo duplications in unrelated individuals.

**Conclusion:** Results corroborates the role of the following genes to the aetiology of CL±P: GLI2, PKP1, MKX, PVRL1, CDH1 and TYMS. 22 genomic segments not previously associated with CL±P were also identified, contributing to a more general framework of the CNV role in CL±P, evidencing the complex aetiology of the condition and the need for biological evidence of all CNVs appointed here and in the literature.

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**P11.030****Identification of de novo variants in nonsyndromic cleft lip with/without cleft palate patients using whole exome sequencing**

N. Ishorst<sup>1,2</sup>, K. U. Ludwig<sup>1,2</sup>, D. Drichel<sup>1</sup>, S. Sivalingam<sup>1,2</sup>, A. C. Böhmer<sup>1,2</sup>, L. Götz<sup>1</sup>, M. Alblas<sup>1,2</sup>, K. Keppler<sup>1,2</sup>, S. L. Mehrem<sup>1,2</sup>, K. Köhler<sup>1,2</sup>, A. Maaser<sup>1,2</sup>, B. Braumann<sup>5</sup>, M. Martini<sup>6</sup>, M. Knapp<sup>7</sup>, M. M. Nöthen<sup>1,2</sup>, T. Becker<sup>8,9</sup>, E. Mangold<sup>2</sup>;

<sup>1</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany,

<sup>2</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>3</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>4</sup>Department of Orthodontics, University of Bonn, Bonn, Germany, <sup>5</sup>Department of Orthodontics, University of Cologne, Cologne, Germany, <sup>6</sup>Department of Maxillo-Craniofacial Surgery, University of Bonn, Bonn, Germany, <sup>7</sup>Institute of Medical Biometry Informatics and Epidemiology, University of Bonn, Bonn, Germany, <sup>8</sup>German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany, <sup>9</sup>Institute for Community Medicine, University of Greifswald, Greifswald, Germany.

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations and has a multifactorial etiology. To date, a number of common risk variants have been identified for nsCL/P, explaining about 24% of the genetic heritability. It has been suggested that some of the remaining genetic liability might be explained by rare *de novo* mutations, which is supported by the frequent observation of sporadic nsCL/P cases and a higher nsCL/P recurrence risk in offspring compared to parents.

We performed whole exome sequencing (WES) in 50 sporadic nsCL/P cases of Central European ancestry and their unaffected parents. Exome capture was performed using SureSelect<sup>XT</sup> Human All Exon V5 and libraries were sequenced using 1x125bp paired-end sequencing. Raw data were processed using the default BWA/GATK v.3.4 pipeline including VQSR and the genotype refinement workflow. Annotation of the possible *de novo* events was done using ANNOVAR.

We identified 158 high-confidence *de novo* variants of which 59 were either missense or nonsense variants which did not map to regions of segmental duplications.

Validations were performed on all variants with a quality-by-depth  $\geq 4$ , excluding variants with bad WES reads in the region of interest. So far, we confirmed all 27 residual variants, including their *de novo* status.

Our preliminary results show the presence of *de novo* mutations in nsCL/P patients. We are currently performing further confirmation and follow-up studies investigating the causality of the true *de novo* variants. These results will be presented at the conference.

**P11.031****Sequencing the GRHL3 coding region reveals rare truncating mutations and the first common susceptibility variant for nonsyndromic cleft palate**

E. Mangold<sup>1</sup>, A. C. Böhmer<sup>1,2</sup>, N. Ishorst<sup>1,2</sup>, A. Hoebel<sup>1,2</sup>, P. Gültape<sup>1,2</sup>, H. Schuenke<sup>1,2</sup>, J. Klamt<sup>1,2</sup>, A. Hofmann<sup>1,2</sup>, L. Götz<sup>3</sup>, R. Raff<sup>4</sup>, P. Tessmann<sup>1,2</sup>, S. Nowak<sup>1</sup>, H. Reutter<sup>1,2,4</sup>, J. Stavusis<sup>5</sup>, M. Ishida<sup>6</sup>, R. Seselyte<sup>6</sup>, G. E. Moore<sup>6</sup>, M. M. Nöthen<sup>1,2</sup>, G. Borck<sup>7</sup>, K. A. Aldhorae<sup>8</sup>, B. Lace<sup>9</sup>, P. Stanier<sup>6</sup>, M. Knapp<sup>9</sup>, K. U. Ludwig<sup>1,2</sup>;

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany, <sup>3</sup>Department of Orthodontics, University of Bonn, Bonn, Germany, <sup>4</sup>Department of Neonatology and Pediatric Intensive Care, Children's Hospital, University of Bonn, Bonn, Germany,

<sup>5</sup>Medical Genetics and Mitochondrial Research Group; Latvian Biomedical Research and study Centre, Riga, Latvia, <sup>6</sup>Institute of Child Health; University College London, London, United Kingdom, <sup>7</sup>Institute of Human Genetics, Ulm University, Ulm, Germany,

<sup>8</sup>Orthodontic Department, College of Dentistry, Thamar University, Thamar, Yemen,

<sup>9</sup>Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany.

The most frequent subtypes of orofacial clefts are nonsyndromic cleft lip with or without cleft palate (nsCL/P) and nonsyndromic cleft palate only (nsCPO), both considered multifactorial. A common syndromic form of clefting is Van der Woude syndrome (VWS) where patients have CL/P or CPO, often but not always associated with lower lip pits. Recently, ~5% of VWS patients were identified with mutations in the grainy head-like 3 (GRHL3) gene. To investigate GRHL3 in nonsyndromic clefting, we evaluated 672 European patients with apparently nonsyndromic clefts (576 nsCL/P, 96 nsCPO) sequencing the GRHL3 coding region. We identified four novel truncating GRHL3 mutations in nine patients from four families, two of them *de novo*. All individuals harboring mutations had exclusively nsCPO. Most interestingly, in nsCPO patients we identified a higher minor allele frequency for rs41268753 (0.099), compared to controls (0.049;  $P=1.24\times 10^{-2}$ ). This association was replicated in nsCPO/control cohorts from Latvia, Yemen and the UK ( $P_{\text{combined}}=2.63\times 10^{-5}$ ;  $P_{\text{allelic}}=2.5$  (95% CI 1.6-3.7)), and reached genome-wide significance in combination with imputed data from a GWAS in nsCPO triads ( $P=2.73\times 10^{-9}$ ). Notably, this variant is not associated with nsCL/P ( $P=0.45$ ). Rs41268753 encodes the highly conserved p.Thr454Met (GERP = 5.2) which numerous in silico prediction programs denote as dele-

terious, has a CADD score of 29.6 and increases protein binding capacity in silico. Thus, our targeted sequencing approach has identified the first common genetic risk factor for nsCPO, and the first gene with rare mutations and a common risk variant for nsCPO in the coding region.

### P11.032

#### Copy Number Variations (CNVs) in patients with multiple malformations and intellectual disability

R. Staneva<sup>1</sup>, S. Hadjidekova<sup>1</sup>, M. Pancheva<sup>1</sup>, D. Avdjieva-Tzavella<sup>2</sup>, B. Rukova<sup>1</sup>, Z. Hammoudeh<sup>1</sup>, O. Antonova<sup>1</sup>, V. Damyanova<sup>1</sup>, M. Mihaylova<sup>1</sup>, R. Tincheva<sup>2</sup>, D. Toncheva<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria,

<sup>2</sup>Department of Clinical genetics - University Pediatric Hospital, Medical University of Sofia, Sofia, Bulgaria.

**Introduction:** In recent years array CGH analysis seems to have been established as an invaluable tool for diagnostics in the field of dysmorphology and intellectual disability. The data gathered the last decade allowed to further refine the interpretation of results and helped to discriminate pathogenic from normal variants.

**Materials and Methods:** 81 patients with multiple malformations, intellectual disability and normal karyotype were included in the study. Peripheral blood sample was collected from every participant after signed written informed consent. Array CGH analysis was performed on Agilent ISCA 4x44k v2.0 oligo-arrays by manufacturer's protocol. Arrays were scanned on Agilent G2505A scanner. Data was analyzed by Bluefuse Multi software v4.2. Variants were further classified based on size, frequency, type, gene content and function into four groups - benign, probably benign, VUS and pathogenic.

**Results:** 281 CNVs were identified. Average number of CNVs per patient is 3,5. Pathogenic CNVs (n=13) were found in 13,6% (n=11) of our patients. Among pathogenic CNVs deletions were 69,2% (n=9), while duplication represented 30,8% (n=4). Pathogenic CNVs sized greater than 1mb represent 76,9% (n=10). 30,8% (n=4) of pathogenic variations were considered irrelevant to the phenotype. Benign CNVs (n=118) and probably benign CNVs (n=110) were respectively identified in 69,1% (n=56) and in 72,8% (n=59) of the patients in our cohort. VUS (n=39) were identified in 35,8% (n=29) of patients.

**Conclusion:** Our study enriched the available data on the copy number variations in Bulgarian population and helped to reclassify some of the VUS as probably benign.

### P11.033

#### Severe Cockayne syndrome or COFS? (Or both?)

J. Behunova, F. Laccone;

Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria.

**Background/Aim:** The authors report on a case of severe form of Cockayne syndrome (CS) with a mutation in ERCC6-gene, that had been described before by COFS (cerebro-oculo-facio-skeletal) syndrome, and discuss a delineation between these two entities.

**CaseReport:** A boy was born in term with a low-normal birth size (2500/48/32) and without cataract or arthrogryposis. Postnatally proceeded severe feeding difficulties (PEG), severe growth retardation (-5,5 SD) and microcephaly (-6,6 SD). In his age of 3,5 y he could not sit and stand alone and showed no speech. His face was not typical „old-looking“, but showed some dysmorphia and extreme dental caries. The trunk was hypotonic, extremities hypertonic, with mild contractures of elbows and knees. Other findings presented cryptorchism, general brain atrophy (MRI), no photosensitivity and no retinopathy. DNA: Homozygote mutation in the ERCC6-gene (c.2047C>T, p.Arg683\*)

**Discussion:** A patient with the same mutation published as a COFS syndrome showed low-normal birth size, congenital arthrogryposis and cataracts with sunken eyes, postnatally severe feeding difficulties, seizures, severe developmental and growth delay with microcephaly (-5 SD) and notable photosensitivity. On the other hand, a few patients with other mutations in ERCC6-gene had been referred as severe CS or CS/COFS, because their congenital symptoms (IUGR, microcephaly, cataract, arthrogryposis) and absent neurological development fulfilled also the definition of COFS.

**Conclusion:** Severe Cockayne syndrome and COFS share highly overlapping features and the same molecular-biochemical characteristics, thus it might be helpful to overcome the historical separation and to recognize them as one entity.

### P11.034

#### Further evidence of the c.314G>A (p.Arg105Gln) mutation in the SMARCE1 gene as a rare cause of Coffin-Siris syndrome

M. Sánchez Soler<sup>1</sup>, I. Valenzuela<sup>2</sup>, L. Fernández<sup>3</sup>, E. Vallespin<sup>3</sup>, T. Vendrell<sup>3</sup>, M. Ballesta-Martínez<sup>1</sup>, V. López-González<sup>1</sup>, V. Fernández-Montaño<sup>3</sup>, F. Nieto<sup>3</sup>, K. Ibañez<sup>4</sup>, J. Silla-Castro<sup>4</sup>, A. del Pozo<sup>4</sup>, F. Santos-Simarro<sup>5</sup>.

<sup>1</sup>Sección de Genética Médica, Servicio de Pediatría, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, <sup>2</sup>Genética Clínica, Área de Genética Clínica i Molecular, Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>3</sup>Genómica Estructural y Funcional, INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>4</sup>Bioinformatics section, INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>5</sup>Genética Clínica, INGEMM, Hospital Universitario La Paz, Madrid, Spain.

**Introduction:** Mutations in several components of the BAF complex have been identified in Coffin-Siris syndrome (CSS), a rare congenital malformation syndrome characterized by developmental delay, coarse facial features and hair and digital abnormalities. Most patients harbour mutations in ARID1B gene (60-70%) but at least mutations in another 5 genes of the complex have been detected. To date, SMARCE1 mutations account for only 2% of the cases, with just two variants reported: c.218A>G (p.Tyr73Ser) in two patients and c.314G>A (p.Arg105Gln) in one patient and his affected mother (the latter of uncertain pathogenicity).

**Material and methods:** A cohort of 18 unrelated patients with features suggestive of CSS has been screened using a custom next generation sequencing panel (BAF\_v1.1 Roche NimbleGen) including the genes of the BAF complex as well as other interacting genes.

**Results:** We present two additional patients in whom the variant p.Arg105Gln in SMARCE1 has been detected, confirmed to be *de novo* in one case, while results of parental testing in the second will be presented in due course. Clinical details of the patients as well as comparison to previously reported patients will be presented.

**Conclusions:** Despite lack of functional studies, our results support the pathogenicity of this variant and provide further insight into the clinical manifestations of these patients. The p.Arg105Gln mutation in SMARCE1 seems to be a rare recurrent mutation responsible of CSS and should be included in the molecular screening of these patients.

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### P11.035

#### Clinical features and mutation spectrum of Coffin-Siris syndrome in Malaysia: A case series

W. P. Ong<sup>1</sup>, M. A. M. Haniffa<sup>1</sup>, G. S. Ch'ng<sup>1</sup>, Y. Tsurusaki<sup>2</sup>, N. Miyake<sup>2</sup>, N. Matsumoto<sup>2</sup>, W. T. Keng<sup>1</sup>;

<sup>1</sup>Department of Genetics, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia, <sup>2</sup>Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

**Introduction:** Coffin-Siris syndrome(CSS) is a rare disorder characterised by intellectual disability, distinctive facies, hypoplastic/aplastic 5<sup>th</sup> fingernails and/or toenails and growth retardation. Pathogenic variants in ARID1A, ARID1B, SMARCA4, SMARCB1, SMARCE1 of the SWI/SNF complex are known to cause CSS.

**Method:** Between January 2013 and July 2015, 17 patients with clinical diagnosis of CSS underwent whole exome sequencing(WES) in search of the SWI/SNF complex genes.

**Results:** We report 12 individuals (5 males,7 females) with molecular confirmation. All had developmental delay, dysmorphism with coarse facies, thick lower lips, hirsutism and hypoplastic nails. Five patients had microcephaly; 8 had failure to thrive; 9 had short stature; 2 had secundum ASD. Cases with non-typical features would also be highlighted. WES identified heterozygous mutations in ARID1B in 10 probands, and in SMARCB1 in 1 patient. Interestingly, a boy with features more in-keeping with CSS had a mutation in SMARCA2, the gene associated with Nicolaides-Baraitser syndrome, highlighting the many similarities of these two syndromes. All were novel mutations. Seven were *de novo*, one child was adopted, four others have yet to be determined. The ARID1B mutations included 4 frameshift insertions, 3 frameshift deletions, 2 nonsense mutations and 1 splice site mutation. The SMARCB1 and SMARCA2 mutations were missense mutations. The child with SMARCB1 seemed the worst affected with severe growth retardation and cognitive impairment.

**Conclusions:** Mutations in ARID1B are the predominant cause for CSS in Malaysia. Due to overlapping phenotypes with Nicolaides-Baraitser syndrome and chromosomal abnormalities, molecular confirmation directs accurate genetic counselling and expands the phenotypic spectrum.

**P11.037****Additional Clinical Findings in two sisters with LAMM syndrome**

*M. Basdemirci<sup>1</sup>, A. G. Zamani<sup>1</sup>, S. Sener<sup>2</sup>, M. Tassoker<sup>2</sup>, H. Cetmili<sup>2</sup>, A. Zamani<sup>3</sup>, M. S. Yildirim<sup>1</sup>;*

<sup>1</sup>*Department of Genetics, Meram Medical Faculty, Necmettin Erbakan University, KONYA, Turkey, <sup>2</sup>Department of Radiology, Faculty of Dentistry, Necmettin Erbakan University, KONYA, Turkey, <sup>3</sup>Department of Chest Disease, Meram Medical Faculty, Necmettin Erbakan University, KONYA, Turkey.*

Microtia and Microdontia (LAMM) syndrome is a rare condition, although its prevalence is unknown. Approximately a dozen affected families have been identified. It is characterized by bilateral sensorineural congenital deafness with labyrinthine aplasia, microtia and microdontia. Minor dysmorphic signs such as long face, micrognathia, antverted ears and skin tags on the upper part of the auricle can be observed in the patients. It is an autosomal recessive condition and associated with homozygous or compound heterozygous mutations in the FGF3 gene.

A 29-year-old woman was admitted with microdontia, oligodontia and sensorineural hearing loss signs to the genetics department. Bilateral microtia with skin tag on the upper part of the left auricle as well as antverted ears were observed in physical examination. Keratoconus was observed in ophthalmologic examination MRI of her inner ear showed bilateral complete labyrinthine aplasia. A CT of the chest revealed a dilated azygos vein. Her sister also had the same symptoms. Parents were first degree cousins. Pedigree analysis showed autosomal recessive trait. Both the patient and her sister were diagnosed with LAMM syndrome.

In this report, we describe two sisters with autosomal recessive LAMM syndrome characterized by three major findings: complete labyrinthine aplasia, microtia and microdontia. Additional clinical findings, dental abnormalities, follow-up, management and mutation types of FGF3 gene will be discussed in detail.

**P11.038****Identification of Congenital Diaphragmatic Hernia genes by Whole Exome Sequencing**

*M. Kammoun<sup>1</sup>, P. Brady<sup>1</sup>, H. Van Esch<sup>1</sup>, J. Deprest<sup>2</sup>, K. Devriendt<sup>1</sup>, J. R. Vermeesch<sup>1</sup>;*

<sup>1</sup>*Department of Human Genetics KU Leuven, leuven, Belgium, <sup>2</sup>Department of Obstetrics and Gynecology, University Hospitals Leuven, leuven, Belgium.*

The genetics of Congenital Diaphragmatic Hernia (CDH) is poorly understood. We hypothesize that Whole Exome Sequencing (WES) application will advance our understanding of genes involved in CDH pathogenesis.

We performed WES on 13 patients from 9 different families. Dominant and recessive models were taken into account for variants calling in all cases. We identified pathogenic variants in known CDH causal genes: ZFPM2, KMT2D and PORCN. In addition, we observed probably pathogenic variants in 3 genes thus far not yet known to cause CDH, including PIGN in a fetus with complex bilateral CDH.

Similarly to the previous reports, ZFPM2 mutations are the most incriminated in CDH and are also observed with a reduced penetrance and a variable expressivity in our families. This gene is a GATA4 activity modulator and is subsequently important for diaphragm and heart development. PORCN gene lesions cause of X-linked Focal Dermal Hypoplasia that was previously considered to be embryonic lethal in male. Interestingly, we are the first to report PORCN variant in non-mosaic males. Defective PORCN, leads to an impairment of WNT trafficking through the cell which affects the downstream genes dependent on this pathway. PIGN is involved in Glycosylphosphatidylinositol anchor biosynthesis. Prior to our report, PIGN mutations were described in association with multiple malformations but seldom with CDH which we suggest to result from a more severe loss of function. As for the new candidate genes, they are known to cause X-linked Intellectual disability and the confirmation of their implication in diaphragm development is ongoing.

**P11.039****Cytogenetic study in children with congenital heart defects and multiple congenital anomalies: ten years of experience**

*A. Boglis<sup>1</sup>, F. Tripone<sup>1</sup>, C. Banescu<sup>1,2</sup>;*

<sup>1</sup>*University of Medicine and Pharmacy of Tîrgu Mureş, Tîrgu Mureş, Romania, <sup>2</sup>Genetic Department of Emergency Clinical County Hospital Tîrgu Mureş, Tîrgu Mureş, Romania.*

Introduction: Chromosomal abnormalities often are detected among children with congenital heart defects (CHDs) when associate multiple congenital anomalies (MCA), with an estimated prevalence of 6-13 cases of CHDs per 1000 live births. The aim of this study was to determine the presence of chromosomal abnormalities detected by conventional cytogenetic analysis among children with CHDs and MCA.

**Material and Methods:** We report a retrospective study of 160 children with CHDs and MCA who were referred to the Genetic Department of Emergency Clinical County Hospital Tîrgu Mureş, Romania, between 2006 and 2015. All patients were clinically evaluated for the presence of dysmorphic features and congenital anomalies. Karyotype analysis was performed in all patients from fresh peripheral blood.

**Results:** Among the 160 children identified with CHDs, 55.6% females, with age ranging from 1 day to 15 years, 65 had a normal karyotype. Chromosomal abnormalities were observed in 95 patients (59.4%), 84 (88.4%) were numerical (70 patients with +21, 5 with +13, 3 with +18, 2 with 45,X, 1 with 47,XY, and 3 polisomy X) and 11 (11.6%) structural [2 with der(21;22) and der(15;21), 3 with del(5p), del(13q), del(X)(q11), 2 with i(18q), i(18p), inv(9), r(4)]. The most frequent type of CHD associated with chromosomal abnormality were ventricular and secundum atrial septal defects.

**Conclusions:** Our findings showed that conventional cytogenetic analysis is still useful in understanding the etiology of CHDs and multiple congenital anomalies. Furthermore, molecular cytogenetic techniques is needed for an accurate diagnosis, when karyotype is normal.

**P11.040****Array CGH studies in postnatal diagnosis of 45 cases with multiple congenital malformations including gastrointestinal tract defects**

*E. Obersztyń<sup>1</sup>, A. Kukowska-Kaźmierczak<sup>2</sup>, B. Wisiowielska-Kowalnik<sup>1</sup>, M. Bartnik<sup>1</sup>, M. Smyk<sup>1</sup>, M. Kędzior<sup>1</sup>, J. Castaneda<sup>1</sup>, N. Beznakow<sup>1</sup>, P. Własienko<sup>1</sup>, R. Śmigiel<sup>1</sup>, K. Źak<sup>1</sup>, E. Sawicka<sup>1</sup>, B. Nowakowska<sup>1</sup>;*

<sup>1</sup>*National Institute of Mother and Child, Warsaw, Poland, <sup>2</sup>Medical University Wrocław, Wrocław, Poland.*

**Introduction:** Gastrointestinal tract malformations represent 5 -10% of congenital defects among newborns. Esophageal atresia (EA), tracheoesophageal fistula (TEF) and anal atresia are the most common, comprising 89% of cases. Chromosomal aberrations have been reported in 6 -10% patients with GI defects whereas the etiology of most cases remains unknown. Array CGH is currently offered as a rapid method for identification of genomic changes with potential role in the etiology of birth defects. Our study's aim was to evaluate the prevalence of pathogenic chromosomal aberrations in neonates with multiple congenital anomalies (preferentially gastrointestinal tract malformations) with or without dysmorphic features.

**Patients and methods:** 45 patients were qualified for aCGH studies according to clinical inclusion criteria in the years 2013-2015. The investigation was carried out using the oligoarray set (CytoSure ISCA 60k v2 OGT, UK). Parental origin studies using FISH / aCGH were also performed.

**Results:** We present a summary of our results including detailed clinical data, type, localization and size of aberrant changes as well as their parental origin. Copy number changes were identified in 8/45 (18%) patients. There were 4 deletions (10q22.3q23.1mat; 6q22.3q23.3mat; 15q11.2mat; 17q11.2mat), 3 duplications (10p15.3p11.21(de novo); 2p23.1 (?); 15q24mat) and one case of supernumerary der(22), as a result of maternally inherited translocation (11;22). To our knowledge, anorectal atresia has not been previously reported in 10q22.3 deletion syndrome. Genotype-phenotype considerations are presented.

**Conclusion:** Our results confirm the utility of aCGH as a first - tier test in multiple congenital malformations.

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**P11.041****A missense mutation in the NIPBL gene isoform A cause a mild and atypical familiar case of Cornelia de Lange Syndrome**

*B. Puisac<sup>1,2</sup>, L. Ström<sup>3</sup>, C. Baquero-Montoya<sup>4,1</sup>, T. Visnes<sup>5</sup>, M. Gil-Rodríguez<sup>1</sup>, M. Hernández-Marcos<sup>1,2</sup>, E. Teresa-Rodrigo<sup>1,2</sup>, P. Gómez-Puertas<sup>5</sup>, Q. Pan-Hammarström<sup>3</sup>, I. Bueno<sup>6,2</sup>, F. J. Ramos<sup>1,6,2</sup>, J. Pié<sup>1,2</sup>;*

<sup>1</sup>*University of Zaragoza, Zaragoza, Spain, <sup>2</sup>CIBERER, Zaragoza, Spain, <sup>3</sup>Karolinska Institute, Stockholm, Sweden, <sup>4</sup>Hospital Pablo Tobón Uribe, Medellín, Colombia, <sup>5</sup>Center of Molecular Biology „Severo Ochoa“, Madrid, Spain, <sup>6</sup>University Hospital „Lozano Blesa“, Zaragoza, Spain.*

Cornelia de Lange syndrome (CdLS) is a congenital developmental disorder characterized by facial dysmorphia, growth retardation, limb malformations and intellectual disability. Approximately 60% of patients have pathological variants in the NIPBL gene and many show the CdLS classical phenotype. We report on two brothers with atypical mild CdLS phenotype who have a missense mutation in NIPBL [c.8387A>G; p.(Tyr2796Cys)]. Both patients have brachycephaly, synophrys, long lashes, mild intellectual disability and an unusual broad bulbous nose. Interestingly, this last feature is described in CdLS patients with mutations in the HDAC8 gene. The mutation identified in our patients is located at the last exon (E47) of the NIPBL gene and it

only affects to its isoform A. In order to better understand the genotype-phenotype correlation, we performed "in vitro" studies with the brothers' fibroblasts that revealed a sensitivity to radiation similar than controls and much lower than other CdLS patients with mutations in NIPBL. Moreover, pyrosequencing studies of different tissues of both cases ruled out the presence of somatic mosaicism. Overall, these results suggest that the position of the variant at the end of the NIPBL gene, that alters only the isoform A of the protein, is likely the cause of the atypical mild phenotype of the two brothers. This work has been funded by ISCIII-FIS (Ref. PI12/01318) and Gobierno Aragón (Ref. B20).

#### P11.042

#### Defining Cornelia de Lange syndrome type 4 phenotype: A young boy with RAD21 mutation

P. Marin Reina<sup>1</sup>, M. Roselló Piera<sup>2</sup>, C. Orellana<sup>2</sup>, F. Martínez<sup>2</sup>, P. Ortega<sup>3</sup>, A. Pérez Aytés<sup>4</sup>;

<sup>1</sup>Neonatal Research Unit. Hospital UyP La Fe, Valencia, Spain, <sup>2</sup>Genetics Department.

Hospital UyP La Fe, Valencia, Spain, <sup>3</sup>Pediatric Nephrology Unit. Hospital UyP La Fe, Valencia, Spain.

**Introduction:** Mutations in RAD21 have been associated to a mild phenotype of Cornelia de Lange Syndrome (CdLS-4). We present a patient with a complex malformative pattern and a novel mutation in RAD21.

**Patient description:** The patient was referred to the genetics clinic at the age of 2 years. He was born at 41 weeks to a 31 year old G2P2 mother and a 31 year old father. Birth weight: 3.620kg. After birth, hypospadias, preauricular tags and dysplastic kidneys were diagnosed. On the physical examination, his weight was at 82th centile (13,5kg), height at 6th centile (83cm) and OFD<1th centile (43cm, -3,79SD). He had bilateral clinodactyly of 5th finger; absence of distal interphalangeal crease in 3th-4th fingers, limitation to elbows extension (radial luxation), ocular refractive defect and mild speech delay. Follow-up: Perthes disease. Chronic renal failure (Renal transplant at 4½ year old). He attends normal school with special support for speech (IQ: 75).

A study of next generation sequencing genetics with a custom panel of 1274 genes associated to neurodevelopmental disorders showed that our patient had a mutation in exon 2 of RAD21 gene (c.68G>A (p.Trp23Ter)) resulting in a premature stop codon. This mutation is absent in both parents.

**Discussion:** Very few patients with CdLS-4 have been reported. Comparing our patient with the previously reported, long philtrum, thick and arched eyebrows, short middle 5th finger phalanges, radial head anomalies and mild developmental deficit emerge as the more common features. More patients are needed to an accurate delimitation of CdLS-4 phenotype.

#### P11.043

#### Multiple systems anomalies syndrome caused by a complex rearrangement resulting in a 7 Mb deletion at 11q24 with two adjacent duplications in 11q23.3-q24.1 and 11q24.3-q25 - a case of Jacobsen syndrome?

R. M. Zechi-Ceide<sup>1</sup>, N. M. Kokitsu-Nakata<sup>1</sup>, L. P. Maximino<sup>2</sup>, M. B. Rafacho<sup>3</sup>, A. C. V. Krepischi<sup>4</sup>, C. Rosenberg<sup>4</sup>, A. Richieri-Costa<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo (HRCA-USP), Bauru, Brazil, <sup>2</sup>Department of Speech Language and Hearing – Bauru Dental School, University of São Paulo, Bauru, Brazil, <sup>3</sup>Post-Graduation Program in Rehabilitation Sciences, Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo (HRCA-USP), Bauru, Brazil, <sup>4</sup>Department of Genetics and Evolutionary Biology, Biosciences Institute, University of São Paulo, São Paulo, Brazil.

Orofacial cleft is the most common craniofacial anomaly, which can occur isolated or part of the clinical spectrum of hundreds of highly heterogeneous syndromes. In many instances to establish the accurate diagnosis is a great challenge, mainly in countries where the access to molecular tests is restricted. Here, we reported a 9 years old Brazilian girl with short stature, macrocephaly, coarse facies, deep set eyes, midface hypoplasia, prominent jaw, cleft palate, delayed of eruption of teeth, short neck, sensorineural hearing loss, recurrent pneumonia, eczema, progressive alopecia, multiple spots, motor delay, speech delay, severe language impairment, learning difficulties and intellectual disability. Karyotype and cranial MRI were normal. Hematological tests showed anisocytosis and microcytosis. Array-CGH detected a complex rearrangement at 11q24, with a deletion of 7 Mb in 11q24.1-24.4 flanked by two duplications: a 3,9 Mb duplication in 11q23.3-q24.1, and a duplication of a 5,7 Mb segment in 11q24.3-q25. The deleted segment contains 40 genes, including the genes BSX, NRGN, ETS-1, FLI-1 and ARHGAP32, responsible for most significant morbidity problems in Jacobsen syndrome. There is an important overlap between the clinical signs of our patient and the Jacobsen syndrome; despite this, the whole of the clinical manifestations

in our patient is unique, suggesting a hitherto not unreported new syndromic picture. We considered that genes mapped within the duplicated regions (~60 genes) probable are contributing to phenotype, encompassing genes already related to cleft palate and ectodermal dysplasia. The accurate diagnosis in this patient has led to specific investigations, resulting a more effective management.

#### P11.044

#### Reverse phenotyping of a patient with CRIP1 gene mutation and further delineation of the associated phenotype

B. Demeer<sup>1</sup>, A. Dadban<sup>2</sup>, P. Vabres<sup>3</sup>, A. Varenterghem<sup>4</sup>, J. Thevenon<sup>5</sup>, D. Bremond-Gignac<sup>6</sup>, J. Rivière<sup>5</sup>, C. Thauvin-Robinet<sup>7</sup>, L. Olivier-Faivre<sup>7</sup>;

<sup>1</sup>genetics department/EA4666, Amiens, France, <sup>2</sup>dermatology department, Amiens, France, <sup>3</sup>dermatology department, Dijon, France, <sup>4</sup>Pediatric gastroenterology department, Amiens, France, <sup>5</sup>molecular genetics department, Dijon, France, <sup>6</sup>Ophthalmology department, Amiens, France, <sup>7</sup>genetics department, Dijon, France.

We report on a 3 ½ year old boy, with prenatal onset growth deficiency (height : -4SD), microcephaly (OFC : -3.5 SD), transient neonatal pancytopenia, facial dysmorphism, feeding difficulties, developmental and speech delay, global hyperlaxity, significant sleep disturbance, and genital, ocular and extremities anomalies. He also presented with generalized pigmentation anomalies, and signs of ectodermal dysplasia. When last seen for follow-up at 5 years old, he had developed epilepsy. Array CGH (Agilent 60k), cytogenetic diagnosis of chromosomal breakage syndrome and metabolic screening were negative. Whole exome sequencing performed found a homozygous frame-shift mutation of the CRIP1 gene, recently described as a novel primordial dwarfism gene (Shaheen et al, 2014). The mutation (c.132delA), described as "probably pathogenic", was confirmed by sanger sequencing. Both healthy consanguineous parents were proved to be carrier in the heterozygous state.

Few available clinical data of the 2 described patients shows very similar clinical appearance with strikingly facial dysmorphism, growth deficiency, microcephaly, psychomotor delay, and ocular and extremities anomalies. Mottled hypopigmentation is noted in the older patient described.

This description is an example of "reverse phenotyping". The first description of the CRIP1 gene by Shaheen et al, helped us to reach a diagnosis in our patient. Nevertheless considering this new case the term of "primordial dwarfism" seem to be too restrictive. For instance, cutaneous signs are specific in our patient. Reports of additional patients will help to further delineate the associated phenotype of this unique syndrome.

#### P11.045

#### Another case of de novo microdeletion 6q13-q15

I. Micle<sup>1</sup>, L. Kornejeva<sup>1</sup>, I. Inashkina<sup>2</sup>, B. Lace<sup>2</sup>;

<sup>1</sup>Childrens' Clinical University Hospital, Riga, Latvia, <sup>2</sup>Biomedical Research and Study Center, Riga, Latvia.

Microdeletion of 6q13-q15 is rare, less than 30 patients have been reported in the literature. Patients suffer from intellectual disability and a distinctive pattern of major and minor anomalies. Here we report a patient with this microdeletion with aim to further delineate this microdeletion syndrome. The patient is a 6 months old Caucasian boy, born to healthy unrelated parents after IVF pregnancy. Diagnostic amniocentesis was done in 16th gw with subsequent FISH analysis for the most common aneuploidies due to the biochemical trisomy screening abnormalities. The results were normal and the pregnancy continued. US revealed agenesis of corpus callosum in the 21st gw and megacystis and dystopic right kidney in the 34th gw. Cesarean section was performed in the 37th gw, because of progressive oligohydramnios and breech presentation.

At birth the child was edematous and hypotonic, he had respiratory distress and tendency to hypoglycemia. Kidney hypoplasia and hydronephrosis were secondary to the posterior urinary valve. Brain MRI confirmed complete agenesis of corpus callosum and gyrus cinguli, colpocephaly, smaller cerebellar hemispheres and inferior vermis. He had umbilical and inguinal hernias, bilateral cryptorchidism. Presenting minor anomalies were up-slanted palpebral fissures, low-set ears, long philtrum, micrognathia, narrow high palate, and wide halluces.

aCGH (CGHMT- 4x180K) was performed and revealed a de novo microdeletion arr6q13q15(7,470,262,0-8,899,132,7)x1.

At 6 months the boy has psychomotor retardation, generalized hypotonia, strabismus, and nystagmus. He developed renal failure during episode of infection and peritoneal dialysis was initiated, which was interrupted when his renal function improved on clinical recovery.

**P11.046****A partial deletion(Xp)/duplication(Xq) case due to maternal pericentric inversion(X) confirmed by microarray**

*O. Boz<sup>1</sup>, B. Sarikepe<sup>1</sup>, E. Ceylan<sup>1</sup>, G. Gonen<sup>1</sup>, E. Tepeli<sup>1</sup>, N. Semerci<sup>1</sup>, F. Duzcan<sup>1</sup>, O. Kirbiyik<sup>2</sup>, B. Ozyilmaz<sup>2</sup>, G. Bagci<sup>1</sup>;*

<sup>1</sup>Pamukkale University, Department of Medical Genetics, Denizli, Turkey, <sup>2</sup>Tepecik Training and Research Hospital, Department of Medical Genetics, Izmir, Turkey.

Carrying a parental pericentric inversion can cause deleted/duplicated offsprings. According the literature, del(Xp)/dup(Xq) is a rare chromosomal aberration in males due to maternal pericentric inv(X).

A seven days old male patient was referred us because of dysmorphic features. He was born at 35 weeks gestation age by C/S because of oligohydramnios. Physical examination findings were narrow biparietal diameter, broad nasal root and nasal bridge, depressed nasal tip, short columella, long, flat philtrum, thin upper lip and retromicrognathia. The patient's karyotype was 46,XY. We performed subtelomeric FISH analysis because of non-syndromic dysmorphic features. Two signals for Xqter were detected on each tip of X chromosome, but no signal was detected for Xpter. His microarray analysis revealed that there were 3.7Mb deletion on Xp22.33p22.31 which contains 9 OMIM genes, 2.5Mb deletion on Xp22.33 pseudoautosomal region which contains 23 OMIM genes, 2 Mb duplication on Xq28 which contains 52 OMIM genes and 270Kb duplication on Xq28 pseudoautosomal region which contains 3 OMIM genes. Chromosomal analysis from both parents were performed for possible pericentric inversion of X chromosome and another chromosomal aberrations. Paternal karyotype was normal, but his mother had pericentric inversion of X chromosome which was confirmed by subtelomeric FISH analysis. His brother also had pericentric inversion(X). We present this case in order to contribute to the literature. It is important to provide genetic counseling that pericentric inversion carrier parents have risk of deleted/duplicated offsprings and affected children.

Muss B, Schwanitz G. Characterization of Inversions as a Type of Structural Chromosome Aberration. *Int J Hum Genet* 2007;141-161

**P11.047****Patient With 22q11.2 Microdeletion and Atypical Clinical Features**

*V. Šliužas<sup>1,2</sup>, Ž. Maldžienė<sup>1,2</sup>, B. Burnyte<sup>1,2</sup>, V. Kučinskas<sup>1,2</sup>, A. Utkus<sup>1,2</sup>;*

<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius, Lithuania,

<sup>2</sup>Centre for Medical Genetics, Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania.

We report on a girl with prominent expressive language impairment, microcephaly, dental, ocular, anorectal anomalies and dysmorphic features. Array-CGH analysis has revealed microdeletion of chromosome 22 spanning ~2.5 Mb at 22q11.21q11.22 (20,098,507-22,556,733 hg19). FISH analysis has confirmed de novo microdeletion origin. Huge variety of different size deletions in this particular site as well as high density of the genes in chromosome 22 forms variety of phenotypes for the DiGeorge, VCFS and related syndromes. Clinical features of our patient distinguish from DiGeorge syndrome, because deletion does not include HIRA and TBX1 genes. Moreover, the deletion detected in our patient genetically and phenotypically distinguishes from previously reported central 22q11.2 deletions. Renal and urogenital anomalies that are common in patients with central 22q11.2 deletion have not been observed in our patient. Additionally, our patient has dental anomalies including hypodontia, abnormal shape and enamel hypoplasia of the central incisor which have not been reported in patients with similar deletions. The distal breakpoint of the deletion coincide with common central 22q11.2 microdeletion breakpoint while the proximal breakpoint of the deletion is atypical and is located more proximally than in previously reported patients with central 22q11.2 microdeletion. Our case confirms the statement that deletions of 22q11.2 region do not represent a single clinical entity but vary depending on the specific mediating low copy repeats and the intervening gene content and emphasizes array-CGH method as the most powerful diagnostic tool.

**P11.048****A new case of distal 22q11.2 microdeletion of region LCR22 E-F**

*J. M. Carbonell-Perez<sup>1</sup>, M. E. Sanchez-Gutierrez<sup>1</sup>, J. Saenz-Hurtado<sup>1</sup>, E. Galan-Gomez<sup>2</sup>, P. Mendez-Perez<sup>2</sup>;*

<sup>1</sup>Hospital Infanta Cristina, Badajoz, Spain, <sup>2</sup>Hospital Materno Infantil, Badajoz, Spain.

Introduction: There are different 22q11.2 distal microdeletions, depending on the mediating LCRs (D to H). They vary in size and position but in literature they have been considered as a single clinical entity. Recently, a classification has been proposed based on size and gene content that proposes three different entities. They share some clinical features: Developmental delay, intellectual disability and mild dysmorphic features. One of them is a

small deletion of about 700 Kb (LCRs E to F). This small deletion is usually a de novo event, is rare (only five patients reported in literature) and shows a milder phenotype without growth restriction or cardiovascular defects.

Materials and methods: The patient was a 10-year-old boy who was attended in Clinical Genetics Unit because of mild intellectual disability. Both parents were phenotypically normal (mother with hypothyroidism) and he was born after 40 weeks of uneventful pregnancy. He had normal developmental milestones but learning difficulties at school. An array-CGH analysis was performed using a 60K platform (Perkin Elmer). FISH with LSI BCR probe was used in patient and parents.

Results and conclusions: Patient had a deletion of about 700 kb in distal 22q11.2 region (LCRs E to F) that was inherited from an apparently normal father. This deletion is rare and usually a de novo event. We report the second patient with an inherited deletion of this region. Our patient supports that this deletion is characterized by a mild phenotype and can be inherited from an apparently normal parent.

**P11.049****A case with atypical cutaneous finding expand the phenotype of the 22q11.2 distal deletion syndrome**

*S. Ahn, J. Kim, S. Shin, S. Lee, J. Choi;*

*Yonsei University College of Medicine, Seoul, Korea, Republic of*

Introduction: Interstitial deletions of chromosome 22q11.2, including DiGeorge and velocardiofacial syndrome, are the most common microdeletions. Recently, rare 22q11.2 distal deletion syndrome with distinct genomic and clinical features from DiGeorge/velocardiofacial syndrome has been delineated. The 22q11.2 distal deletion syndrome is characterized by developmental delay, pre- and postnatal growth restriction, facial dysmorphism, and mild skeletal anomalies. We report a patient with phenotype compatible with the 22q11.2 distal deletion syndrome and with novel phenotype, cutaneous hemangioma.

Materials and Methods: A 3-year-11-month old male was referred for genetic evaluation for moderate mental retardation and developmental delay. He had short stature, microcephaly, small forehead, high palate, tongue tie, clinodactyly, left cryptorchidism, and large strawberry hemangioma on his trunk. G-banding karyotype using peripheral blood was normal. For screening of common microdeletion syndromes, multiple ligation-dependent probe amplification (MLPA) using P245 microdeletion syndrome probemix was performed.

Results: MLPA showed heterozygous deletion only for one probe site in PPIL2 gene at 22q11.21, which located distal to common deleted region of DiGeorge syndrome. For confirmatory analysis of screening result, subsequent MLPA using P250 DiGeorge probemix showed heterozygous deletion for two additional probe sites located in TOP3B and HIC2 genes. The deletion involved low-copy repeats (LCR) 22-D region, distinct from recurrent deletion of DiGeorge/velocardiofacial syndrome, and contained 51 genes.

Conclusions: We have identified a rare 22q11.2 distal deletion syndrome case with previously unreported cutaneous feature. Our finding can expand our knowledge of the clinical spectrum of the 22q11.2 distal deletion syndrome. MLPA is an easy and sensitive analytical method to diagnose these small-sized microdeletion syndromes.

**P11.050****DNMT3A p.Arg882His somatic mutation recurrently observed in adult acute myeloid leukemia can cause Tatton-Brown-Rahman syndrome when present in germline**

*R. Kosaki<sup>1</sup>, H. Terashima<sup>2</sup>, M. Kubota<sup>3</sup>, K. Kosaki<sup>4</sup>;*

<sup>1</sup>Division of Medical Genetics, National Center for Child Health and Development Hospital, Setagaya, Tokyo, Japan, <sup>2</sup>Division of Neurorogy, National Center for Child Health and Development Hospital, Setagaya, Tokyo, Japan, <sup>3</sup>Division of Neurology, National Center for Child Health and Development Hospital, Setagaya, Tokyo, Japan,

<sup>4</sup>Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan.

DNA methylation plays critical role for both embryonic development and tumorigenesis and is mediated through various DNA methyltransferases. Germline mutations in the de novo DNA methyltransferase DNMT3A cause peculiar form of overgrowth syndrome, Tatton-Brown-Rahman syndrome (TBRs). Somatic mutations in DNMT3A are causally associated with acute myeloid leukemia (AML) and p.Arg882His represents the hotspot. Mutation spectrum in TBRs and AML never overlapped to date. Furthermore, no patients with TBRs have reported to have developed AML. We report a livebirth and survival of an infant with the TBRs phenotype who had the germline de novo DNMT3A mutation at the AML somatic mutation hotspot p.Arg882His. The female patient had a birth weight of 3926 g (+2.2SD) and length of 54 cm (+2.7SD). Characteristic features included hypotonia,

round face, narrow palpebral fissures, ventricular septal defect, umbilical hernia, sacral cyst, Chiari type I anomaly, and intellectual disability. At age 6 years, she had overgrowth with a weight of 29.2 kg (+3.2 SD) and a length of 125.8 cm (+3.1SD) and head circumference of 55cm (+2.8SD). Identification of the same variant (DNMT3A p.Arg 882 His) as both germline mutation associated with TBRS and somatic mutation associated with AML directly links mechanistic basis of the two diseases for the first time. We conclude that mutations responsible for TBRS and those for AML are likely to exert the same mode of action: decreased methyltransferase activity and reduced DNA binding activity. From a clinical standpoint, our observation indicates surveillance for hematologic abnormalities is needed among patients with TBRS.

#### P11.051

#### A novel mutation of TBC1D24 identified in a Turkish family with DOORS syndrome

E. Atli, H. Gurkan, S. Demir Ulusal, Y. Karal, E. I. Atli, H. Tozkir; Trakya University, Edirne, Turkey.

**Introduction:** Deafness, onchodystrophy, osteodystrophy and mental retardation (DOOR or DOORS) syndrome (OMIM 220500) is a rare autosomal recessive disorder of unknown cause. Small or absent nails and hypoplastic terminal phalanges are seen in most individuals. The syndrome is also associated with seizure disorder. Approximately 50 affected individuals have been described in the medical literature. Half of the patients with all clinical features have mutations in TBC1D24.

**Materials and Methods:** A 18-month-old girl case born to a G3P2A1 female at term with NSVD as 2200 gr. Her parents were second degree consanguineous. Microcephaly, short neck, broad forehead, pointed chin, hypertelorism, high narrow palate, preauricular tag, flat nasal bridge, open nose wings, absent fingernails were determined in physical examination. Optometry, EEG and cranial MR results were normal in our patient. The patient had hearing loss, mental retardation and tonic-clonic seizures for 7 times.

**Results:** Cytogenetic analysis revealed 46,XX,inv(9)(p11q13) karyotype. We performed sanger sequencing for TBC1D24 (OMIM 613577) gene with deep intronic in-house designed primers. We identified a homozygous single base alteration c.1415 G>A (p.G428R) in TBC1D24 gene. This mutation was found in the proband's parents.

**Conclusions:** To our knowledge, the c.1415 G>A mutation has not been reported previously. The c.1415G>A was considered to be damaging by SIFT software, probably damaging (score 1.000) by Polyphen, and disease-causing by Mutation Tester and the CADD scaled c-score was 34. Therefore, our finding is considered as the first case report of this mutation.

#### P11.052

#### 6,6 Mb de novo 22q11.1- q11.22 duplication in a patient with anomalous pulmonary venous drainage, intestinal malrotation and pre B acute lymphoblastic leukemia

L. - Cimbalistiene<sup>1,2</sup>, B. Aleksiuniene<sup>1,2</sup>, V. Dirse<sup>3</sup>, I. Tamuliene<sup>4</sup>;

<sup>1</sup>Department of Human and Medical Genetics, Vilnius, Lithuania, <sup>2</sup>Centre for Medical Genetics at Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania,

<sup>3</sup>Hematology, Oncology and Transfusion Medicine Center at Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania, <sup>4</sup>Centre for Pediatric Hematology/Oncology, Childrens Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania.

The 22q11.2 duplication syndrome is an extremely variable disorder ranging from normal to cognitive deficits, dysmorphic facial features, congenital defects and overlaps with some features of DiGeorge/ Velocardiofacial syndromes.

We present a patient with dysmorphic features, congenital defects who was diagnosed with pre-B acute lymphoblastic leukemia (pre-B-ALL). The proband is the first child of non-consanguineous parents was born with birth weight 3900 g, height 59 cm. Anamnestically he had the surgeries of anomalous pulmonary venous drainage and the intestinal malrotation. At the age of 4, pre-B ALL and secondary hyperparathyroidism were diagnosed. The following dysmorphic facial features were noted: mild ptosis, broad flat nose, down slanting palpebral fissures, opened mouth. His psychomotor development was normal.

Chromosome analysis revealed a mosaic karyotype 47, XY,+mar[85]/46,XY[15]. To further determination the chromosomal origin of the marker chromosome whole-genome SNP array was carried out and identified a de novo 6.6 Mb gain at chromosome region 22q11.1-q11.22. The additional genome-wide analysis of patient bone marrow confirmed germline 22q11.1-q11.22 duplication, and revealed other somatic aberrations - 9.8 Mb in size deletion and hyperdiploidy with trisomies of 6, 11, 14,

17, 18 chromosomes. The mosaic duplication encompasses a region containing over 90 genes including the TBX1 gene, which overexpression might be responsible for the patient's phenotype. SEPT5, GP1BB, DGCR2, CLTCL1, CDC45L genes, which are responsible for the cell cycle division, cells proliferation and could be leukemia-related. We do hypothesize, that this duplication may influence the clonal events in the bone marrow and increase the predisposition to pre-B-ALL.

#### P11.053

#### Duplication, partial duplication and triplication of 9p in children and their impact on phenotype (4 cases)

K. Adamová<sup>1</sup>, V. Curtísová<sup>1</sup>, P. Čapková<sup>1</sup>, M. Holzerová<sup>2</sup>, M. Jarošová<sup>2</sup>, A. Šantavá<sup>1</sup>, J. Srovnal<sup>3</sup>;

<sup>1</sup>Department of Medical Genetics, University Hospital and Palacky University, Olomouc, Czech Republic, <sup>2</sup>Hemato- Oncology Clinic, University Hospital and Palacky University, Olomouc, Czech Republic, <sup>3</sup>Institute of Molecular and Translational Medicine, University Hospital and Palacky University, Olomouc, Czech Republic.

We present four cases of aberrations of the short arm of chromosome 9 detected postnatally in children and the impact on their phenotype.

We describe two cases of full trisomy 9p, one case of partial trisomy 9p and one case of tetrasomy 9p. In two patients isochromosome i(9p) and isodicentric chromosome idic(9q) were present. Only one aberration arose as a consequence of balanced chromosomal translocation in mother all other cases were the novo. Patients were examined by G- banding, FISH and CGH or array CGH.

Trisomy 9p is a rare chromosomal syndrome (Rethoré sy) originally reported in the medical literature in 1970 and first proposed as a distinct syndrome in 1975. It is one of the most frequent autosomal rearrangements compatible with long survival rate. It arises either as a consequence of balanced CNA in parents or the novo and is associated with mental retardation and characteristic facial dysmorphism. There is some discrepancy in the literature regarding the consistency of clinical features present with relation to the size of the duplicated segment.

Tetrasomy 9 is a very rare chromosomal disorder first described in 1973 (Ghymers et al). Isochromosome or isodicentric chromosome is present in most cases. Phenotype features of tetrasomy 9p are variable among affected individuals including varying degrees of growth retardation, abnormal facial features, and mental retardation. Death in the neonatal period is common.

#### P11.054

#### Interstitial deletion and duplication of 15q25.2-q25.3

C. B. Mueller-Hofstede, N. Lichey, U. E. Siebers-Renelt, P. Wieacker, A. Röpke; Institut für Humangenetik, Muenster, Germany.

We report on a girl with a complex chromosomal rearrangement in the region of 15q25.2-q25.3 detected by array CGH analysis. Clinically, the girl presented at the age of 23 months because of global developmental delay and failure to thrive. Birth-weight, length and head circumference were in the normal range. After birth, difficulties of feeding and hypotonia were observed. Hereafter she developed moderate psychomotor retardation. At presentation she showed short stature, microcephaly, dystrophy and unspecific craniofacial dysmorphism.

Chromosomal analysis generated inconspicuous results. Array CGH analysis uncovered a complex rearrangement with an interstitial 1,6 Mb deletion in 15q25.2 adjacent to a 607,9 kb duplication in 15q25.2-q25.3. The imbalances were confirmed by qPCR in the child and excluded in both parents. FISH analysis using two BAC clones revealed a small paracentric inversion (inv(15)(q25.2q25.3)) in the mother.

Isolated deletions in 15q25.2 have already been described in individuals with intellectual disability. Inverted duplications associated with terminal deletions resulting in the loss of the subtelomeric region are reported for different chromosomes (Zuffardi et al. 2009). To the best of our knowledge an interstitial inverted duplication with deletion has been described only once in 1p36.1 (Milosevic et al. 2014). In contrast to our patient there was no predisposing chromosomal abnormality in both parents.

We report on a rare case of interstitial inverted duplication with deletion of 15q25.2-q25.3 resulting from a maternal paracentric inversion. This underlines the necessity of an additional cytogenetic workup of the parents including FISH analysis for the detection of predisposing structural rearrangements.

#### P11.055

#### The 3rd W522X mutation in EIF2AK3 gene from Turkey: a new patient with Wolcott-Rallison syndrome

A. Unal, T. Bahsi, A. Bakir, E. Percin;

Gazi University, Medical Genetics Department, Ankara, Turkey.

**Introduction:** Wolcott-Rallison syndrome (WRS), also known as multiple

epiphyseal dysplasia with early-onset diabetes mellitus is a rare autosomal recessive multisystemic disorder. Its characteristic clinical features are permanent neonatal or early infancy insulin-dependent diabetes and later onset skeletal dysplasia. Other frequent clinical manifestations are hepatic and renal dysfunction, mental retardation, cardiac abnormalities, exocrine pancreatic dysfunction, primary hypothyroidism and neutropenia. Although WRS is a rare disease, it is the most common cause of permanent neonatal diabetes mellitus (PNDM) in consanguineous families. The exact frequency of this rare syndrome is unknown because patients may die before showing the characteristic features of the syndrome.

Case: This report presents a case of WRS in an 8-year-old girl with neonatal diabetes and short stature whose elder brother died of diabetes mellitus at 2-months-old age before having a molecular diagnosis.

Results: We found a homozygous W522X mutation in *EIF2AK3* gene in our patient which was only found in another two unrelated Turkish families.

Discussion: W522X mutation in *EIF2AK3* gene seems to be confined to Turkey and may be a common mutation in WRS patients from this country. In this paper, we compare the clinical features of the patients having W522X mutation with other patients reported to date. Except the characteristic features as diabetes mellitus and epiphyseal dysplasia, all the WRS patients show extensive phenotypic variability that correlates poorly to genotype which suggests that there is no correlation between any specific mutation and the clinical manifestation.

#### P11.056

#### Paternal uniparental disomy as an etiologic cause of Ellis van Creveld syndrome

M. C. D'Asdia<sup>1</sup>, V. G. Naretto<sup>2</sup>, B. Torres<sup>1</sup>, G. Botta<sup>3</sup>, A. Sciarrone<sup>4</sup>, A. Onori<sup>1</sup>, I. Torrente<sup>1,5</sup>, B. Marino<sup>6</sup>, L. Bernardini<sup>7</sup>, A. De Luca<sup>1</sup>

<sup>1</sup>CSS-Mendel Institute, Rome, Italy, <sup>2</sup>S.C. Genetica Medica - U.A.O. - U. Città della Salute e della Scienza, Torino, Italy, <sup>3</sup>Servizio di Anatomia Patologica, Presidio Ospedaliero O.I.R.M.-Sant'Anna A.O.-U Città della Salute e della Scienza, Torino, Italy, <sup>4</sup>Centro di Ecografia e Diagnosi prenatale, A.O.-U Città della Salute e della Scienza, Torino, Italy,

<sup>5</sup>Department of Experimental Medicine, Sapienza University, Rome, Italy, <sup>6</sup>Department of Pediatrics, Sapienza University, Rome, Italy.

Ellis-van Creveld (EvCS; OMIM 225500) syndrome is an autosomal recessive disorder characterized by a disproportionate limb dwarfism, chondroectodermal dysplasia, congenital heart disease, postaxial polydactyly, and dysplastic fingernails and teeth, resulting from loss-of-function mutations in EVC or EVC2 genes. We report a fetus presenting with micromelia, narrow thorax with short ribs, and polydactyly of hands on ultrasound scan at 21 weeks' gestation. The pregnancy was terminated at this stage and autopsy revealed brachydactyly, postaxial polydactyly of both hands, short ribs, abnormally short long bones, and dysplastic iliac wings. Fetal tissue was collected fresh in order to perform molecular assays. Sequence analysis of the 21 coding exons of EVC gene revealed that the fetus was homozygous for a frameshift mutation, NM\_153717.2:c.1388del;p.(Thr463Serfs\*37). DNA from parental blood samples was analyzed to confirm the carrier status of the parents. Analysis of the samples showed that the mutation identified in the fetus was not present in the mother. The simplest explanation for this would have been a maternally inherited deletion. SNP-array testing was performed to determine the gene copy number and analyze the haplotype in the 4p16.2 region in which EVC gene is located. The results showed no deletion, but the haplotype revealed the uniparental isodisomy of chromosome 4. Characterization of the homozygous nature of the mutation through parental genotyping and SNP-array analysis allowed us to confirm that uniparental isodisomy of the paternal chromosome 4 carrying the mutated EVC gene played an etiologic role in the disease.

#### P11.057

#### Mutations in DYNC2LI1 cause Ellis-van Creveld phenotype

M. Niceta<sup>1</sup>, M. C. Digilio<sup>2</sup>, K. Margiotti<sup>1</sup>, M. L. Dentici<sup>2</sup>, F. R. Lepri<sup>3</sup>, F. Pantaleoni<sup>4</sup>, S. Barresi<sup>1</sup>, A. Palma<sup>4</sup>, P. Bencivenga<sup>1</sup>, A. De Luca<sup>4</sup>, A. Novelli<sup>3</sup>, E. Bertini<sup>5</sup>, B. Dallapiccola<sup>6</sup>, M. Tartaglia<sup>1</sup>

<sup>1</sup>Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, <sup>2</sup>Medical Genetic Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, <sup>3</sup>Laboratory of Medical Genetics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, <sup>4</sup>IRCCS-Casa Sollievo della Sofferenza, Mendel Institute, Rome, Italy, <sup>5</sup>Department of Neurosciences, Unit of Molecular Medicine, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, <sup>6</sup>Scientific Directorate, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

DYNC2LI1 encodes a component of the dynein-2 complex of intraflagellar transport (IFT), which is crucial for proper ciliogenesis. Biallelic, probably inactivating, mutations in DYNC2LI1 have previously been reported to cause a broad phenotypic spectrum, including Jeune syndrome and diverse phenotypes within the spectrum of "short-rib thoracic" dysplasias. We previously described two sisters exhibiting features fitting Ellis-van Creveld syndrome but also presenting with hydrometrocolpos and partial atrioven-

tricular canal defect. Here, we performed whole exome sequencing in one of the two sisters, and identified compound heterozygosity for inactivating mutations in the DYNC2LI1 gene. Sanger analyses confirmed these variants in both affected sisters and in a third member of the family, who displayed an overlapping phenotype. Our finding indicates that inactivating DYNC2LI1 mutations can underlie Ellis-van Creveld phenotype.

#### P11.058

#### Alternations in genes expression microarray in esophageal atresia tissues - the role of cytokine-cytokine receptor interaction and Rho cell motility signaling pathways

A. Jakubiak, P. Leszczynski, P. Skiba, M. M. Sasiadek, R. Smigiel;  
Medical University, Wroclaw, Poland.

Esophageal atresia (EA) with or without tracheoesophageal fistula belongs to common congenital anomalies. EA may occur as isolated (IEA) or syndromic (SEA). EA etiology is complex and still unexplained. It is suggested that multifactorial mechanism combined with epigenetic factors play role in EA etiology.

The material for study was RNA extracted from esophageal tissue acquired from newborns with esophageal atresia. The control group was RNA extracted from esophageal tissues taken from aborted fetuses and stillborn neonates without congenital defects. In first step the gene expression profiling in IEA and SEA vs control was done. The expression analysis was performed by microarray methods (Agilent SurePrint G3 Human GE). After the functional analysis of signaling pathways (by KEGG database and DAVID) two pathways were chosen - cytokine-cytokine receptor interaction and Rho cell motility signaling pathway and nine genes (CCL2, LIF, TNF, TNFRSF6B, LIMK1 ARHGEF1, ARHGEF11, TLN1, VCL) were analyzed by real-time PCR (The LightCycler® 480 System, Roche). Microarray examination was performed on 26 tissues, real-time PCR was done in group of 20 tissues (10 of IEA and 10 of SEA). Statistical analysis after Real-time PCR was done by Rest2009 Software, Qiagen GmbH.

In microarray we identified about 4800 up and down regulated probes between IEA and controls and about 5000 up and down regulated probes between SEA and controls. About 2300 probes occurred both in IEA and SEA groups. Analysis after real-time PCR showed down-regulation for ARHGEF1 gene in IEA vs. control and for LIF gene in SEA vs. control.

#### P11.059

#### Trio-based whole exome sequencing reveals de novo causative mutations in esophageal atresia

R. Zhang;

Institute of Human Genetics, University of Bonn, Bonn, Germany.

Esophageal atresia with or without tracheoesophageal fistula (EA/TEF) occur approximately 1 in 3500 live births representing the most common malformations of the upper digestive tract comprising five anatomical subtypes classified on the basis of the location and the type of anastomosis that exists between the trachea and the esophagus. The etiology is yet poorly understood.

Here we performed whole exome sequencing in 34 case-parent-trios with EA/TEF to identify disease causing de novo events. WES was performed using a 100bp paired-end read protocol as per the manufacturer's recommendations on an Illumina HiSeq2000 sequencer. Data analysis was done by bwa-aln, gatk, samtools and the de novo probability tool DeNovoGear using the VARBANK pipeline (CCG, Cologne).

Using standard filter criteria our preliminary analysis identified 28 apparent de novo variants in 17 unrelated patients. Of these we confirmed 26 de novo variants with Sanger sequencing. Of these CHD7 has been previously associated with syndromic EA/TEF phenotypes. Currently we evaluate the remaining variants for their possible involvement in the development of EA/TEF in large EA/TEF patient cohorts.

#### P11.060

#### Utilization of whole exome sequencing for undiagnosed diseases in Hong Kong

C. Mak, H. Chung, Y. Chu;

Department of Paediatrics, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: In the past few years, undiagnosed diseases programs worldwide have had increasing success in finding the etiology of pediatric diseases. However, in Hong Kong, whole exome sequencing (WES) is not funded by the government and there is yet to be a large collaborated effort to utilize this technology. We piloted the use of WES in pediatric patients sup-

ported by research funding and collaboration with overseas centers.

Materials and Methods: 70 pediatric patients with undiagnosed diseases were recruited through the genetic clinic, and singleton DNA samples were sent for WES in collaboration with an overseas laboratory. After initial analysis of the variant based on the phenotype, segregation analysis for candidate pathogenic variants was performed locally.

Results: Using a singleton WES strategy followed by targeted parental Sanger sequencing, pathogenic variants were found in over 30% (n=21) of the patients, including conditions recently identified with mutations identified in causative genes e.g. ASXL3, DDX3X, PIGO, PURA etc. We shall present some illustrative patients and address the challenges of implementing this technology in our local healthcare system.

Conclusions: With limited resources, we piloted the application of WES for undiagnosed pediatric diseases in Hong Kong. Importantly our strategy of singleton WES followed by targeted parental Sanger sequencing has made it less costly compared to trio-based WES and yet achieving a diagnostic rate comparable to those reported overseas.

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## P11.061

### Novel Mendelian disorder associated with the impaired function of the RNA exosome

*N. Di Donato<sup>1,2</sup>, T. M. Neumann<sup>3</sup>, A. Kahlert<sup>4,4</sup>, B. Klink<sup>1</sup>, K. Hackmann<sup>1</sup>, I. Neumann<sup>5</sup>, B. Novotna<sup>6</sup>, J. Schallner<sup>6</sup>, I. A. Glass<sup>2,7,8</sup>, S. E. Parnell<sup>9</sup>, A. Benet-Pages<sup>3</sup>, A. M. Nissen<sup>3</sup>, W. Berger<sup>10</sup>, J. Altmüller<sup>11</sup>, H. Thiele<sup>11</sup>, B. H. F. Weber<sup>12</sup>, E. Schrock<sup>1</sup>, W. B. Dobyns<sup>2,7,13</sup>, A. Bier<sup>14</sup>, A. Rump<sup>1</sup>;*

<sup>1</sup>Institute for Clinical Genetics, TU Dresden, Dresden, Germany, <sup>2</sup>Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, United States,

<sup>3</sup>Medical Genetics Center, Munich, Germany, <sup>4</sup>Department of Congenital Heart Disease and Pediatric Cardiology, University Hospital of Schleswig-Holstein, Kiel, Germany,

<sup>5</sup>MVZ Prof. Neumann, Munich, Germany, <sup>6</sup>Sozialpaediatrisches Zentrum, Klinik fuer Kinder und Jugendmedizin, Universitaetsklinikum Dresden, Dresden, Germany,

<sup>7</sup>Division of Genetic Medicine, Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, WA, United States, <sup>8</sup>Division of Genetic Medicine, Department of Medicine, University of Washington, Seattle, WA, United States,

<sup>9</sup>Department of Radiology, Seattle Children's Hospital, University of Washington, Seattle, WA, United States, <sup>10</sup>Institute of Medical Molecular Genetics, University of Zurich, Schlieren, Switzerland, <sup>11</sup>Cologne Center for Genomics, Cologne, Germany, <sup>12</sup>Institute of Human Genetics, University of Regensburg, Regensburg, Germany, <sup>13</sup>Departments of Pediatrics and Neurology, University of Washington, Seattle, WA, United States, <sup>14</sup>Gemeinschaftspraxis für Humangenetik, Dresden, Germany.

Retinitis pigmentosa in combination with hearing loss can be a feature of different Mendelian disorders. We describe a novel syndrome caused by biallelic mutations in the "exosome component 2" (EXOSC2) gene. Three individuals from two unrelated German families presented with a previously undescribed disorder encompassing childhood myopia, early onset retinitis pigmentosa, progressive sensorineural hearing loss, hypothyroidism, short stature, brachydactyly, recognizable facial gestalt, premature aging, and mild intellectual disability. Whole exome sequencing revealed homozygous and compound heterozygous mutations in EXOSC2 in all three patients who shared a large 6.7 Mb rare haplotype on chromosome 9 encompassing EXOSC2.

EXOSC2 encodes the "ribosomal RNA-processing protein 4" (RRP4) - one of the core components of the RNA exosome. The RNA exosome is a multi-protein complex that plays key roles in RNA processing and degradation. One of the discovered amino acid substitutions p.Gly30 by Valin located within the N-terminal domain of EXOSC2 would change the inter-atomic distance between the mutated EXOSC2 residue and neighboring amino acids of EXOSC4 and, therefore, destabilize EXOSC2-EXOSC4 interaction. A similar mechanism was proposed for the recurrent mutation p.Gly31Ala in the NT domain of EXOSC3. Intriguingly, the EXOSC2-associated phenotype shows only minimal overlap with the previously reported diseases associated with mutations in the RNA exosome core component genes EXOSC3 and EXOSC8. Taken together, we report on a novel condition that is caused by altered RNA exosome function and expands the spectrum of clinical consequences of impaired RNA metabolism.

## P11.062

### Any direct role of FANCA in mitochondria?

*R. Bottega<sup>1</sup>, S. Ravera<sup>2</sup>, M. Faleschini<sup>1</sup>, R. Bortul<sup>1</sup>, E. Cappelli<sup>3</sup>, C. Dufour<sup>3</sup>, M. Zwayer<sup>1</sup>, A. Savoia<sup>1</sup>;*

<sup>1</sup>Department of Medical Sciences, University of Trieste, Trieste, Italy, Trieste, Italy,

<sup>2</sup>Biochemistry Lab., Department of Pharmacy, University of Genova, Genova, Italy, Genova, Italy, <sup>3</sup>Clinical and Experimental Hematology Unit, G. Gaslini Children's Hospital, Genoa, Italy, Genoa, Italy.

Fanconi anemia (FA) is a genetic disorder characterized by chromosomal instability, congenital abnormalities, pancytopenia, and predisposition to cancer. Though they play a role in DNA repair processes, the FA proteins are likely to have other functions. Indeed, FA cells show defective mitochondria associated with increased levels of reactive oxygen species and apoptosis. In order to provide insights into the role of FANCA, one of the FA proteins, we investigated its localization and found that it resides not only in the nucleus and the cytoplasm but also in mitochondria. We also analysed its expression of in cells derived from patients. Unexpectedly, proteins affected by amino acid substitutions are stably expressed in the cytoplasm, suggesting that they could be hypomorphic, exerting some role in the mitochondria. Consistent with this hypothesis, the mitochondrial phenotype is less severe in cells carrying one or two missense alleles than in those completely lacking FANCA (null cells).

The energetic and respiratory metabolism of mitochondria was restored or partially restored when null FANCA cells were transfected with wild type or missense mutant forms of FANCA, respectively, further strengthening the hypothesis of a role of FANCA in mitochondria.

The potential role of the FA proteins in mitochondria has long been neglected despite evidence for defects of this organelle in FA cells. Finding that FANCA localizes in the mitochondrion leads the way into investigations that could explain the increased redox status and apoptosis observed in FA.

## P11.063

### Screening of Fanconi Anemia associated genes reveals BRIP1 mutation frequency may be higher than expected in patients from Turkey

*G. Bagirova, G. Toksoy, S. Avci, Z. Uyguner, U. Altinoglu, S. Basaran, H. Kayserili;*

*Department of Medical Genetics, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey.*

Introduction: FA is recessively inherited chromosomal instability syndrome characterized by bone marrow failure, congenital malformations and cancer predisposition. Pathogenic variants of FANCA are attributed to 60-70%, while BRIP1 is associated in approximately 2% of the FA patients.

Material and method: Targeted gene panel is design to cover all the coding exons of 17 FA genes plus flanking exon-intron regions up to 10 bp. Genetic analysis is performed on Ion Torrent PGM platform, and detected mutations and/or alterations considered to be pathogenic are verified by Sanger and screened in family for segregation with inheritance pattern.

Results: 6 out of 14 alleles found to carry mutations. Two different homozygous mutations (c.894-2A>G and c.4261-2A>C) in FANCA and one compound heterozygous [(c.205+5G>T)+(c.761\_764delAGCA)]mutations in BRIP1 gene are identified, accounting to three DEB positive patients.

Conclusions: 72 different mutations of BRIP1 gene are known and out of 14 are involved in germ line bi-allelic FA. c.761\_764delAGCA identified in our study is novel and attained to be the second deletion that is reported in the context of FA, striking the DEAD-2 domain of the encoding protein that is different than the former (c.2255\_2256delAA), damaging the HELIC domain.

The results of the limited number of individuals tested in this study, reveals that the DEB positivity assures the definitive diagnosis and if the DEB test is ignored, mutation detection rate is calculated to be 42%, when presently known FA genes are analyzed, in which that the 28% holds for FANCA and the 14% holds for BRIP1 associated mutations.

## P11.064

### A de novo 13q31.1q.31.2 deletion spanning MIR17HG associated with Feingold syndrome type 2 and keratoconus

*F. Sirchia<sup>1,2</sup>, E. di Gregorio<sup>1</sup>, B. Pasini<sup>1,2</sup>, E. Grosso<sup>1</sup>, A. Brusco<sup>1,2</sup>, D. Carli<sup>1,2</sup>, G. Gai<sup>1</sup>, V. Naretti<sup>1</sup>, A. Zonta<sup>1</sup>, G. Restagno<sup>1</sup>;*

*Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Torino, Italy, <sup>2</sup>University of Torino, Department of Medical Sciences, Turin, Italy.*

We report on a 58-year old woman with microcephaly, mild dysmorphic features, bilateral keratoconus, digital abnormalities, short stature and mild cognitive delay. The phenotype was suggestive for Feingold syndrome type 2, an autosomal dominant disorder characterized by a variable combination of microcephaly, digital abnormalities, short stature and mild-to-moderate intellectual disability. MIR17HG is the gene known to cause the disease (FGLDS2, MIM614326).

Karyotype analysis showed a deletion on chromosome 13q, further defined by Array-CGH to span a 17.3-Mb region on 13q31.1q31.2, including the MIR17HG gene. The deletion was proved to be de novo by real-time qPCR. Feingold type 2 is a very rare Mendelian syndrome, described in few patients worldwide. Our proband shows a never reported association between FGLDS2 and keratoconus. We noted that the 17.3-Mb deletion on chromosome 13q partially overlap with the keratoconus 7 locus, identified by linkage analysis on Ecuadorian families (MIM614629). Thus we suspect that haploinsufficiency of a gene nearby MIR17HG, defined by the overlapping segment between our deletion and the linkage region is causing keratoconus. Nine genes are included in this segment. Among these, the best candidates are MBNL2 and IPO5. MBNL2 encodes a muscleblind protein responsible for terminal differentiation of muscle and photoreceptor tissues. IPO5 encodes a protein nuclear transport and it is expressed in human cornea. In conclusion, we describe a possible contiguous gene syndrome phenotypically characterized by Feingold syndrome type 2 and keratoconus, due to a large deletion on 13q overlapping MIR17HG and a still to be identified gene for keratoconus.

#### P11.065

#### A novel synonymous mutation in FGFR1 causes Hartsfield syndrome

C. Courage<sup>1,2</sup>, J. Neidhardt<sup>3</sup>, F. Dallèves<sup>2</sup>, J. Lemke<sup>2</sup>;

<sup>1</sup>Folkhälsoinstitutet of Genetics, Helsinki, Finland, <sup>2</sup>Division of Human Genetics, University Children's Hospital, Inselspital, Bern, Switzerland, <sup>3</sup>Institute of Human Genetics, University of Oldenburg, Oldenburg, Germany, <sup>4</sup>Institute of Human Genetics, University of Leipzig, Leipzig, Germany.

Hartsfield syndrome is a rare clinical entity characterized by the triad of holoprosencephaly, ectrodactyly and cleft lip/palate. In addition to these symptoms patients with Hartsfield syndrome can show developmental delay of variable severity, isolated hypogonadotropic hypogonadism (IHH), central diabetes insipidus, vertebral anomalies, eye anomalies and cardiac malformations. Mutations in FGFR1 have been described to cause a wide phenotypic spectrum such as Hartsfield syndrome, hypogonadotropic hypogonadism with or without anosmia, Jackson-Weiss syndrome, osteoglophonic dysplasia, Pfeiffer syndrome and trigonocephaly type 1.

Here we describe a novel synonymous mutation in FGFR1 identified by exome sequencing in two siblings born to non-consanguineous healthy Swiss parents. The male patient presented with lobular holoprosencephaly with a single maxillary incisor, ectro-/syndactyly on both feet, syndactyly on both hands, craniosynostosis of the sagittal suture, delayed puberty and developmental delay. His younger sister was diagnosed with diabetes insipidus, in addition to syndactyly of the right foot and an aortic isthmus stenosis. A cranial MRI showed agenesis of corpus callosum and colpocephaly. The novel synonymous missense mutation c.1029G>A (p.Ala343Ala) FGFR1 detected in both affected siblings, was excluded in DNA extracted from leukocytes of the parents and their healthy sister. Therefore, we assume a gonadal mosaicism or somatic mosaicism including germ cells of this mutation. In one of the FGFR1 isoforms this guanine to adenine substitution is located in a specific splice acceptor region, which we currently are functionally validating *in vitro*.

#### P11.066

#### Fraser syndrome: fetal presentation and prenatal diagnosis from a series of 35 cases

A. Tessier<sup>1</sup>, M. Sarreau<sup>2</sup>, F. Pelluard<sup>3</sup>, G. André<sup>3</sup>, A. Beaufrère<sup>4</sup>, S. Blesson<sup>5</sup>, M. Bucourt<sup>6</sup>, P. Callard<sup>7</sup>, I. Creveaux<sup>8</sup>, P. Dechelotte<sup>9</sup>, T. Frebourg<sup>1</sup>, B. Gilbert-Dussardier<sup>9</sup>, C. Jeanne-Pasquier<sup>10</sup>, F. Guimiot<sup>11</sup>, A. Laquerrière<sup>12</sup>, P. Loget<sup>13</sup>, C. Mechler<sup>14</sup>, M. Perez<sup>15</sup>, P. Tixeront<sup>16</sup>, R. Van Der Luijt<sup>17</sup>, S. Patrix<sup>12</sup>, A. Guerrot<sup>1</sup>;

<sup>1</sup>InsERM U1079, Rouen University and Department of Genetics, Rouen University Hospital, Normandy Centre for Genomics and Personalized Medicine, Rouen, France, <sup>2</sup>Department of Obstetrics, Angoulême Hospital, Angoulême, France, <sup>3</sup>Department of Foetopathology, Bordeaux Pellegrin University Hospital, Bordeaux, France, <sup>4</sup>Department of Pathology, Clermont Ferrand University Hospital, Clermont-Ferrand, France, <sup>5</sup>Department of Genetics, Tours University Hospital, Tours, France, <sup>6</sup>Department of Foetopathology, Jean Verdier Hospital, Bondy, France, <sup>7</sup>Department of Pathology, Tenon Hospital, Paris, France, <sup>8</sup>Department of Molecular Genetic, Clermont Ferrand University Hospital, Clermont-Ferrand, France, <sup>9</sup>Department of Genetics EA3808, Poitiers University and Poitiers University Hospital, Poitiers, France, <sup>10</sup>Department of Pathology, Caen University Hospital, Caen, France, <sup>11</sup>Department of developmental biology, UMR1141, Robert Debré University Hospital and Paris Diderot University, Paris, France, <sup>12</sup>Department of Pathology, Rouen University Hospital, Rouen, France, <sup>13</sup>Department of Pathology, Rennes University Hospital, Rennes, France, <sup>14</sup>Department of Pathology, Louis Mourier Hospital, Colombes, France, <sup>15</sup>Department of Foetopathology, Department of Medical Genetics, Montpellier University Hospital, Montpellier, France, <sup>16</sup>Department of Pathology, Poitiers University Hospital, Poitiers, France, <sup>17</sup>Department of Genetics, Utrecht Medical Center University, Utrecht, Netherlands.

Fraser syndrome (FS) is a rare autosomal recessive malformation disorder described for the first time in 1962. Major criteria are cryptophthalmos, syndactyly, respiratory and urinary tract anomalies. Mutations have been iden-

tified within 3 genes, FRAS1, FREM2 and GRIP1, all involved in mesenchymal-epidermal adhesion during embryonic development. This descriptive study analyzed the prenatal ultrasound and fetal phenotype in 35 cases of FS. Termination of the pregnancy was performed in 24 cases, intra-uterine death was diagnosed in 8 cases, between 16 and 36 weeks of pregnancy, and 3 cases died after birth. All cases presented dysmorphic features with nose and ear dysplasia. Complete or abortive cryptophthalmia and renal anomaly (agenesis, hypoplasia or dysplasia) were present in 34/35 cases, syndactyly in 33/35, bronchopulmonary anomalies in 30/34, genital anomalies in 30/35, and sexual phenotype was frequently difficult to establish. Multiple anomalies were observed in the digestive tract: low set umbilicus (23/34), anal atresia/stenosis (16/33), intestinal malrotation (10/33) and omphalocele (6/35). Ultrasound results were available in 24 cases. Reported anomalies were oligoamnios (18), ascites (8), renal anomalies (19), bronchopulmonary anomalies (11), ophthalmologic anomalies (4), ear dysplasia (2) and syndactyly (2). The fetal and postnatal phenotype of FS is very specific, while ultrasound diagnosis is complicated by the presence of oligoamnios. This study shows that cardinal FS diagnosis criteria are rarely found on prenatal ultrasound. Evidence for low set umbilicus, microptalmia, and genital anomalies should lead to consider the diagnosis of FS.

#### P11.067

#### Frontonasal dysplasia in two girls with EFNB1 gene duplications

I. V. Kanivets<sup>1,2,3</sup>, V. G. Solonichenko<sup>3</sup>, S. N. Stavtseva<sup>4</sup>, F. A. Konovalov<sup>1,2</sup>, D. V. Pyankov<sup>1,2</sup>, S. A. Korostelev<sup>1,2</sup>;

<sup>1</sup>Genomed Ltd, Moscow, Russian Federation, <sup>2</sup>FSBI „Research Centre for Medical Genetics“, Moscow, Russian Federation, <sup>3</sup>Filatov Children's clinical hospital, Moscow, Russian Federation, <sup>4</sup>Z.I. Kruglaya Regional Children's Clinical Hospital, Orel, Russian Federation.

Frontonasal dysplasia occurs in patients with various genomic abnormalities, including chromosomal rearrangements, point mutations and copy number variations (CNVs) affecting single genes. We examined two girls, aged 1 year and 5 months, with developmental delay, frontonasal dysplasia, and other congenital anomalies. In addition to frontonasal dysplasia, the 1-year old girl had hypotonia, deformed low-set ears, hemangioma of upper lip. In the 5-months old girl, additional phenotypic features included plagiocephaly, camptodactyly and umbilical hernia. Chromosomal microarray analysis (CMA) using SNP array (Affymetrix CytoScan 750k) was performed as a first-line test according to ACMG recommendations. Both girls appeared to have a copy number gain of EFNB1 gene: a 1-year old girl had 3 copies (molecular karyotype: arr Xq13.1(67,923,907-68,158,871)x3, size 235 kb), and a 5-months old girl had 4 copies (molecular karyotype: arr Xq13.1(67,863,904-68,457,240)x4, size 693 kb). According to the OMIM database, point mutations and deletions (but no duplications so far) in EFNB1 gene were described in X-linked dominant Craniofrontonasal syndrome (304110) with prevalence in female patients.

The use of CMA allows identification of genes having clinically significant dosage effect associated not only with copy number losses, but with gains as well. This may lead to a revision in the testing strategy for patients with distinctive facial features.

#### P11.068

#### Input of exome sequencing in clinical and molecular characterization of patients referred for Fronto-Nasal Dysplasia

D. Lehalle<sup>1,2,3</sup>, P. Kuentz<sup>3</sup>, J. Thevenon<sup>3,1,2</sup>, D. Bonneau<sup>4</sup>, M. Fradin<sup>5</sup>, M. Gérard<sup>6</sup>, A. Goldenberg<sup>7</sup>, T. Martin-Denavit<sup>8</sup>, A. Masurel<sup>1,2</sup>, M. Rio<sup>9</sup>, S. Whalen<sup>10</sup>, B. Bessière<sup>11</sup>, E. Bieth<sup>12</sup>, V. Cormier-Daire<sup>9</sup>, F. Demurier<sup>5</sup>, P. Edery<sup>9</sup>, D. Geneviève<sup>13</sup>, S. Julia<sup>12</sup>, D. Lacombe<sup>14</sup>, S. Marlin<sup>9</sup>, S. Odent<sup>5</sup>, J. Plaisancié<sup>12</sup>, A. Putoux<sup>9</sup>, C. Thauvin<sup>1,2,3</sup>, L. Faivre<sup>1,2,3</sup>, P. Callier<sup>2</sup>;

<sup>1</sup>Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (TRANSLAD), Centre Hospitalier Universitaire Dijon, Dijon, France, <sup>2</sup>Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs de l'Interrégion Est, Centre Hospitalier Universitaire Dijon, Dijon, France,

<sup>3</sup>Equipe GAD, EA4271, Faculté de Médecine, Université de Bourgogne, Dijon, France, <sup>4</sup>Service de Génétique Médicale, CHU d'Angers, Angers, France, <sup>5</sup>Service de Génétique clinique, CHU de Rennes - Hôpital Sud, Rennes, France, <sup>6</sup>Service de Génétique, Hôpital Clémenceau, CHU de Caen, Caen, France, <sup>7</sup>Service de Génétique, CHU de Rouen - Hôpital Charles Nicolle, Rouen, France, <sup>8</sup>Service de génétique, CHU de Lyon HCL - GH Est - Hôpital Femme Mère Enfant, Bron, France, <sup>9</sup>Département de Génétique, Université Paris Descartes, Sorbonne Paris Cité, Institut IMAGINE UMR\_S1163, Hôpital Necker-Enfants Malades, Paris, France, <sup>10</sup>Hôpital Pitié-Salpêtrière, Département de Génétique et de Cytogénétique, Unité fonctionnelle de génétique clinique, Paris, France, <sup>11</sup>Département d'Histologie-Embryologie et Cytogénétique, Hôpital Necker-Enfants Malades, Paris, France, <sup>12</sup>Département de Génétique, CHU de Toulouse, Toulouse, France, <sup>13</sup>Service de Génétique Médicale, CHU Montpellier, Montpellier, France, <sup>14</sup>Service de Génétique Clinique, CHU de Bordeaux, Bordeaux, France.

Introduction: Fronto-nasal dysplasias (FND) are facial malformations characterized by hypertelorism, median facial cleft and nasal tip anomalies. Their rarity and the lack of molecular basis in most entities explain clinical

and genetic heterogeneity. Mutations in ALX genes family are responsible for autosomal recessive (ALX1, ALX3, ALX4) or dominant (ALX4) FND. Cranio-fronto-nasal syndrome (CFNS), acromelic FND and Teebi syndromes are caused by mutations in respectively EFNB1, ZSWIM6 and SPECC1L. Molecular basis of oculo-auriculo-fronto-nasal (OAFNS), oculo-cerebro-cutaneous (OCCS) and Pai syndromes are not known.

**Materiel and methods:** We report a series of 35 patients referred to sequence ALX genes. Sanger sequencing of ALX1, ALX3 and ALX4 was performed in 14 individuals and identified one ALX3 heterozygous mutation in one patient. Based on the phenotype, we identified 24 FND, 2 CFNS, 5 OAFNS, 2 OCCS, and 2 Teebi syndromes. Sanger sequencing of EFNB1 was performed in both individuals presenting with CFNS. Trio exome sequencing was performed in seven patients.

**Results:** We identified *de novo* EFNB1 mutations in the two CFNS patients, as well as new mutations in known genes (YWHAE, OTX2 and SPECC1L) in patients respectively labeled as FND, OCCS and Teebi syndrome, and a *de novo* variant in a candidate gene in a fetus with FND. Replication cohorts are being sequenced.

**Conclusion:** This series and these results highlight the input of exome sequencing in the identification of molecular basis in syndromes with FND, leading to a better clinical description of these rare entities and allowing to broaden the phenotypic spectrum of known syndromes.

#### P11.069

#### Genetic and genomic analysis in patients affected by Gorham-Stout disease and general lymphatic anomalies

*L. Rodriguez Laguna<sup>1</sup>, M. Crespo<sup>1</sup>, V. Fernandez-Montañó<sup>1</sup>, R. Martín-Arenas<sup>1</sup>, E. Vallespin<sup>1</sup>, A. del Pozo<sup>1</sup>, K. Ibañez<sup>2</sup>, J. Silla-Castro<sup>1</sup>, G. Herranz<sup>2</sup>, G. Oliva-Molina<sup>1</sup>, R. Mena<sup>1</sup>, M. Gomez<sup>1</sup>, I. Rueda-Arenas<sup>1</sup>, J. Nevado<sup>1</sup>, P. Lapunzina<sup>2</sup>, J. López-Gutiérrez<sup>3</sup>, V. Martínez-Glez<sup>2</sup>,*

<sup>1</sup>INGEMM-idiPAZ, Madrid, Spain, <sup>2</sup>INGEMM-idiPAZ-CIBERER, Madrid, Spain,

<sup>3</sup>Department of Pediatric Surgery, Hospital Universitario La Paz, Madrid, Spain.

**Introduction:** Gorham-Stout disease (GSD) and general lymphatic anomalies (GLA) are potentially fatal conditions with significant mortality rates, characterized by lymphatic malformations (LMs) and different patterns of osteolysis. The genetic causes are still unknown, but somatic mosaicism is suspected.

**Methods and Results:** Our database includes 78 GSD and 52 GLA patients. All experiments included paired blood/tissue samples. Genomic dose experiments (Illumina SNP BeadChips) did not detect common UPD/CNV regions (germline or somatic). Mutational screening using NGS experiments (Exome and TruSight One, Illumina), was performed in 18 patients. In-house bioinformatics tools were developed to call somatic variants. A list of 578 candidate genes was obtained—48 were somatic—. No PIK3CA mutations—known to be associated to cystic LMs—were detected, but variations in other four PI3K associated genes were found. As a second step we designed a custom NGS panel with a 1.000X vertical coverage, including the 578 candidate genes as well as PIK3CA associated genes and other vascular/lymphatic genes. We present here a preliminary candidate gene list.

**Conclusion:** Use of NGS—at large reading depths—and specific bioinformatic algorithms for detecting somatic variations in paired blood/tissue samples, is the appropriate methodology for gene discovery in entities caused by mutations in somatic mosaicism form. We have obtained a candidate gene list, including four PI3K associated genes, taking us closer to the cause of GSD/GLA. Our future approach includes the isolation of lymphatic endothelial cell from LMs in GSD/GLA patients as a necessary tool for further genetic and functional analysis.

#### P11.070

#### The eighth patient with Gorlin-Chaudhry-Moss Syndrome

*S. Lanciotti<sup>1</sup>, C. Coco<sup>2</sup>, F. Brancati<sup>3</sup>,*

<sup>1</sup>Section of Genetics, Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy, <sup>2</sup>Altamedica maternal-fetal Medical Centre, Rome, Italy, <sup>3</sup>Section of Genetics, Department of Medical, Oral and Biotechnological Sciences, Gabriele D'Annunzio University, Chieti, Italy.

Gorlin-Chaudhry-Moss Syndrome (GCMS; OMIM%233500), firstly described in 1960 in two sisters, is a neglected condition reported in the medical literature in only seven females. GCMS associates craniostenosis (coronal), ectodermal dysplasia features including hair (diffuse hypertrichosis, synophrys, coarse hair) and teeth (abnormally shaped, hypodontia, microdontia) abnormalities, microphthalmia, conductive deafness, minor congenital heart defects and hypoplastic labia majora. All patients show normal intelligence. Although craniofacial manifestations are characteristic partial overlap with Saethre-Chotzen syndrome exist. We report the eighth case of GCMS diagnosed at the age of 14 years. She was the second child of healthy

unrelated parents born post-term after uneventful pregnancy. At 10 weeks, she was hospitalized for marked craniofacial dysmorphism, cleft palate, bilateral microphthalmia with cataract, interventricular and interatrial septal defects with patent ductus arteriosus. Growth was delayed. Cranial CT scan confirmed craniostenosis involving the coronal sutures. At clinical examination diagnosis was suggested based on craniofacial characteristics including brachycephaly with midface hypoplasia, low frontal hairline, synophrys, microphthalmia, posteriorly angulated ears, bifid nasal tip, prominent columella, dental abnormalities, arched narrow palate with bifid uvula, generalized hypertrichosis and short stature. Left cutaneous syndactyly of toes 2-3 was evident. We requested an audiogram revealing left sided conductive hearing loss. GCMS is exceedingly rare with core features consisting in craniostenosis, ocular and ectodermal derivatives defects. Autosomal recessive inheritance was suggested since the disease recurred in two pairs of sisters. So far, no gene associated to this syndrome exists thus radically limiting diagnosis and reproductive options in affected individuals and their families.

#### P11.071

#### Growth retardation, delayed bone age and mild developmental delay in a patient with microduplication at 2p25.1- case study

*K. Wojciechowska<sup>1</sup>, A. Poluha<sup>1</sup>, B. Wiśniowiecka<sup>2</sup>, B. Nowakowska<sup>1</sup>, J. Kowalczyk<sup>1</sup>,*

<sup>1</sup>Childrens University Hospital, Lublin, Poland, <sup>2</sup>Institute of Mother and Child, Warsaw, Poland.

More than 20 patients have been reported in the literature with duplication of the short arm of chromosome 2. The affected patients had both different sizes of the duplications and they also have different clinical features. Recurrent phenotypic features include growth retardation, delayed bone age and mild developmental delay. We report a 12-year-old female patient with 180 kbp duplication at 2p25.1 which she inherited from her mother. Our patient is noted to have severe growth retardation, delayed bone age and mild developmental delay. Also her mother has relatively low height.

Investigation with whole-genome oligonucleotide microarray CGH analysis which was performed using clinical 60K microarrays from Oxford Gene Technology (CytoSure ISCA v2) revealed duplication spanning of approximately 180 kbp and between breakpoints rs9,955,335 and rs10,136,171 at 2p25.1.

This duplicated region contains GRHL1, KLF11, CYS1 genes as well as a part of the TAF1B gene.

Literature review revealed that GRHL1 gene plays an important role in epithelial development and epidermal differentiation. KLF11 is involved in the regulation of cell growth and differentiation and it is a glucose-inducible regulator of the insulin gene. TAF1B is a component of RNA polymerase I core factor complex.

Our patient is the first reported patient with a duplication of above-mentioned genes and her clinical features conform the symptoms which are present in most of the reported patients with different duplications at the short arm of chromosome 2.

#### P11.072

#### Novel missense FGFR1 mutation in a patient with Hartsfield syndrome

*M. Piotrowicz<sup>1</sup>, A. Sowińska-Seidler<sup>2</sup>, A. Jamsheer<sup>2</sup>, L. Jakubowski<sup>1</sup>,*

<sup>1</sup>Department of Genetics, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland, <sup>2</sup>Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland.

Hartsfield syndrome is a rare genetic disorder characterized by the association of holoprosencephaly (HPE) and ectrodactyly, with or without cleft lip/palate, and variable additional abnormalities. Dominant or recessive FGFR1 loss of function mutations have been recently shown to give rise to Hartsfield syndrome.

In this paper, we report a sporadic case of a male subject with Hartsfield syndrome who presented with ectrodactyly, semilobar HPE, bilateral cleft lip and palate, bilateral vesicoureteral reflux, cryptorchidism, mental retardation, and facial dysmorphism composed of flat facial profile, dysplastic ears, and thin vermillion border. No causative abnormalities were found at the cytogenetic level, including karyotype and 1.4 M array CGH. Upon molecular screening of FGFR1 gene by means of Sanger sequencing, we detected a novel heterozygous missense c.1868A>G (p.Asp623Gly) variant, which was subsequently confirmed to occur as a *de novo* mutational event. The variant was predicted to be damaging to the protein function by the common bioinformatic algorithms used for pathogenicity assessment (MutationTaster2, SIFT, Polyphen2). Additionally, the variant was not annotated in ExAC, EVS or dbSNP databases, however a substitution within the same amino acid position (p.Asp623Tyr) was already described in another case of Hartsfield syndrome.

Our paper provides a detailed clinical presentation of newly identified male individual affected with Hartsfield syndrome and expands the mutational spectrum associated with this extremely rare genetic condition.

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#### P11.073

##### Case report: A novel *CCBE1* gene mutation for Hennekam lymphangiectasia syndrome

F. Harrazi<sup>1</sup>, M. Yavari<sup>1,2</sup>, S. Seyedhassani<sup>1</sup>;

<sup>1</sup>Dr.Seyedhassani medical genetic center, yazd, Iran, Islamic Republic of, <sup>2</sup>Department of Biology, Faculty of Sciences, Yazd University, Yazd, Iran, Islamic Republic of.

Introduction: Hennekam lymphangiectasia syndrome is a rare autosomal recessive condition. Onset is usually in childhood. The syndrome presents with lymphedema, intestinal lymphangiectasia, intellectual deficit and facial dysmorphism. The diagnosis of Hennekam is suspected on the basis of clinical phenotypic features. Phenotypic abnormalities relate to impaired prenatal and postnatal lymphatic flow resulting from mutations in the collagen and calcium-binding EGF-domain 1 (*CCBE1*) during lymphangiogenesis. In this study, we analyzed the DNA sequences of *CCBE1* gene in a patient aged 3 years old with Hennekam syndrome.

Materials and methods: The entire coding regions of the *CCBE1* gene were analyzed for mutations by PCR-based direct DNA sequencing.

Results: One novel homozygous mutation IVS7(+12) A>G at position 640 on *CCBE1* gene has been detected. The heterozygosity of the parents for this IVS7 was confirmed by direct sequence analysis. Bioinformatic analysis confirmed our studies.

Conclusions: This mutation occurs in the deep intronic positions. Activation of an intronic cryptic acceptor site and creation of an intronic ESE site could be created by the mutation. Complementary assessment such as functional study should be considered for suggestion of prenatal diagnosis.

#### P11.074

##### A Turkish girl with Hennekam Syndrome

A. I. Gurler, T. Bahsi, D. Karaer, K. Karaer;

dr ersin arslan Training and Research Hospital medical genetic, gaziantep, Turkey.

Hennekam syndrome is an autosomal recessive syndrome, characterized by generalized lymphatic dysplasia (ie. lymphedema and lymphangiectasia), variable intellectual disability and characteristic dysmorphic features. Recent studies reported patients with Hennekam Syndrome having mutations in *CCBE1* gene.

A 7 years-old girl was referred to our center because of mild intellectual disability and dysmorphic features. She was borned to healthy 2nd degree cousins relative parents. The child had chylous acid, lymphatic congestion and dysmorphic features (bilaterally epicanthus, hypertelorism, flat nasal bridge, full cheeks, flat malar region, long philtrum). A mutation analysis of *CCBE1* gene was requested. The results revealed a previously published sequence variant a missense c.520C>T (p.Cys174Arg) in a homozygous state. The patient we describe here has a lymphatic dysplasia with mild intellectual disability and dysmorphism caused by mutation in *CCBE1*, highlighting the phenotypic variability that can be seen with abnormalities in this gene.

#### P11.075

##### Microdeletions/duplications in patients with holoprosencephaly phenotype

L. A. Ribeiro-Bicudo<sup>1</sup>, N. A. Bergamo<sup>1</sup>, B. S. Maranhão<sup>1</sup>, J. T. Arruda<sup>1</sup>, V. M. Torres<sup>1</sup>, J. Marino<sup>1</sup>, B. F. Gamba<sup>2</sup>, A. R. Costa<sup>3</sup>;

<sup>1</sup>Federal University of Goias, Goiânia, Brazil, <sup>2</sup>Sao Paulo State University, Botucatu, Brazil, <sup>3</sup>Sao Paulo University, Bauru, Brazil.

Holoprosencephaly (HPE) is a malformation sequence in which the cerebral hemispheres fail to separate into distinct left and right halves, resulting in midline structural anomalies of the central nervous system and face. Numerous different heterozygous mutations have been identified in HPE patients, including missense, nonsense, deletion, and frameshift mutations located throughout the gene. In addition, in a recent study of over 200 patients with isolated HPE, 22% were found to have copy number variations (CNVs). However, the majority of etiologies of isolated HPE have yet to be determined.

In the present work, the MLPA technique was performed in 52 individuals within the HPE spectrum who presented negative mutational screening test for the major HPE genes, and anomalies were found in seven cases. These

aberrations included five deletions and two duplication in genes related to HPE. Three individuals had deletions involving SHH on 7q, one individual had a duplication involving SHH on 7q, one individual had a deletion on 18p involving the TGIF1 gene, and another individual had a duplication on 13q involving the ZIC2 gene. Our results also showed that microdeletions/duplications in the major HPE genes SHH, TGIF, and ZIC2 are causative for the frank HPE phenotype, being the SHH the major one (9.5% in SHH, 1.9% in TGIF, and 1.9% in ZIC2). As HPE patients are surviving longer, we believe that an effort to search for CNVs is a valuable tool for genetic counseling when analyses for mutations in the known HPE genes are negative.

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#### P11.076

##### *TBX3* and *TBX5* duplication: a family with an atypical overlapping Holt-Oram/Ulnar-Mammary syndrome phenotype

M. Holder-Espinasse<sup>1</sup>, E. Thomas<sup>1</sup>, A. Davies<sup>2</sup>, N. Timoney<sup>3</sup>, A. Frigola<sup>4</sup>, M. Logan<sup>5</sup>;

<sup>1</sup>Clinical Genetics Department, Guy's Hospital, London, United Kingdom, <sup>2</sup>Genetics laboratories, Guy's Hospital, London, United Kingdom, <sup>3</sup>Plastic Surgery Department, St Thomas Hospital, London, United Kingdom, <sup>4</sup>Cardiology Department, St Thomas Hospital, London, United Kingdom, <sup>5</sup>5. Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Campus, London, United Kingdom.

Holt-Oram syndrome (HOS) is a rare autosomal dominant heart-hand syndrome due to mutations in the *TBX5* transcription factor. A wide spectrum of *TBX5* mutations has been previously reported, mostly resulting in null allele and haploinsufficiency, but some mutations also affect the nuclear localisation of the *TBX5* protein or its interaction with co-factors and downstream targets. *TBX5* duplications have been previously reported in association with atypical HOS phenotypes. Ulnar-Mammary syndrome (UMS) is also a rare autosomal dominant condition due to mutations in the *TBX3* gene. Clinical variability is the rule for both HOS and UMS and incomplete penetrance has often been reported. Contiguous chromosome 12q24 deletions comprising both *TBX5* and *TBX3* genes have been identified but to our knowledge, mirror duplications have never been described.

We report on a large family with at least 10 affected individuals presenting with a 399kb duplication at 12q24.21 identified on array-CGH and comprising *TBX5* and *TBX3* genes, over 3 generations. Patients are presenting with variable limb anomalies involving both the radial and the ulnar rays and interesting cardiac findings such as hypertrabeculation of the left ventricular cavity, possibly in keeping with left ventricular non compaction, tachycardia, persistent arterial duct or aortic stenosis... Additional findings such as accessory nipples were also noted. Fluorescent in situ hybridisation is currently pending to confirm the breakpoints of the 12q24.21 duplication and the underlying mechanism. We will also attempt to look at allele-specific expression and to sequence around the breakpoints to explain the molecular contribution to the clinical findings.

#### P11.077

##### Chitayat Syndrome: hyperphalangism, facial anomalies, hallux valgus, and bronchomalacia results from a recurrent c.266A>G (p.Tyr89Cys) variant in the ERF gene

M. Balasubramanian<sup>1</sup>, J. A. Bernstein<sup>2</sup>, H. Lord<sup>3</sup>, S. Levesque<sup>4</sup>, F. Thuriot<sup>4</sup>, A. Wenger<sup>2</sup>, G. Sillon<sup>5</sup>, H. Guturu<sup>2</sup>, D. L. Sureka<sup>2</sup>, G. Bejerano<sup>2,6,7</sup>, D. Chitayat<sup>8</sup>;

<sup>1</sup>Sheffield Clinical Genetics Service, Sheffield, United Kingdom, <sup>2</sup>Stanford Children's Health, Lucile Packard Children's Hospital, Stanford University School of Medicine, Stanford, CA, United States, <sup>3</sup>Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust, The Churchill Hospital, Oxford, United Kingdom,

<sup>4</sup>Department of Pediatrics, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC, Canada, <sup>5</sup>Department of Medical Genetics, McGill University Health Center, Montreal, QC, Canada, <sup>6</sup>Department of Computer Science, Stanford University, Stanford, CA, United States, <sup>7</sup>Department of Developmental Biology, Stanford University, Stanford, CA, United States, <sup>8</sup>The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital; Division of Clinical Genetics and Metabolism, Department of Pediatrics, The Hospital for Sick Children; University of Toronto, Toronto, ON, Canada.

Background: In 1993, Chitayat et al., reported a newborn with hyperphalangism, facial anomalies, and diffuse bronchomalacia. We have identified 2 more families with similar findings. Characteristic features include bilateral accessory phalanx resulting in shortened index fingers; hallux valgus; facial features including prominent eyes, hypertelorism, depressed nasal bridge and upturned nose; respiratory compromise due to bronchomalacia necessitating ventilatory support; pectus excavatum.

Methods: Trio-based exome sequencing was performed in 3 unrelated families. Putative de novo variants were identified from exome data using DeNovoGear software and validated using Sanger sequencing.

Results: The same variant c.266A>G p.(Tyr89Cys) Refseq NM\_006494.2 in ERF: de novo (Patient 1&2) and inherited from affected father (Patient 3),

was identified. The p.Tyr89Cys is an aromatic polar neutral to polar neutral amino acid change, highly conserved across species and lies within the functionally important ets-domain of the protein. This variant has never been reported (1000 genomes, dbSNP build 144, EVS or EXAC). The recurrent c.266A>G p.(Tyr89Cys) ERF missense variant causes Chitayat syndrome. Discussion: ERF has been shown to suppress ets-induced transformation and be regulated by phosphorylation throughout the cell cycle via ras/MAPK signalling pathway. ERF variants have been associated with complex craniosynostosis. In contrast, none of the patients with the c.266A>G p.(Tyr89Cys) variant have craniosynostosis.

Conclusions: We report the molecular aetiology of Chitayat syndrome consistent with the classical triad of hyperphalangism, characteristic face and respiratory abnormalities. We discuss potential mechanisms for this distinctive phenotype associated with p.Tyr89Cys substitution in ERF and explore why this variant does not present with craniosynostosis.

#### P11.078

#### Recessive inactivating mutations in TBCK, a Rab GTPase-activating protein that modulates mTOR signaling, cause severe infantile syndromic encephalopathy

V. Caputo<sup>1\*</sup>, J. X. Chong<sup>2\*</sup>, I. G. Phelps<sup>2</sup>, L. Stella<sup>3</sup>, L. Worgan<sup>4</sup>, J. C. Dempsey<sup>2</sup>, A. Nguyen<sup>2</sup>, S. Paolacci<sup>1</sup>, V. Leuzzi<sup>5</sup>, R. Webster<sup>6,7</sup>, A. Pizzutti<sup>8</sup>, C. T. Marvin<sup>9</sup>, G. E. Ishak<sup>9</sup>, S. Arden-Holmes<sup>7</sup>, Z. Richmond<sup>9</sup>, M. J. Bamshad<sup>2,10,11</sup>, X. R. Ortiz-Gonzalez<sup>12</sup>, M. Chopra<sup>9,13,14</sup>, D. Doherty<sup>8,11</sup>, M. Tartaglia<sup>8,15</sup>.

<sup>1</sup>Dipartimento di Medicina Sperimentale, Università La Sapienza, Rome, Italy,

<sup>2</sup>Department of Pediatrics, University of Washington, Seattle, WA, United States,

<sup>3</sup>Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, Rome, Italy, <sup>4</sup>Department of Clinical Genetics, Liverpool Hospital, Liverpool, Australia,

<sup>5</sup>Dipartimento di Pediatria e di Neuropsichiatria Infantile, Università La Sapienza, Rome, Italy, <sup>6</sup>T.Y. Nelson Department of Neurology and Neurosurgery, The Children's Hospital at Westmead, Sydney, Australia, <sup>7</sup>Institute of Neuroscience and Muscle Research, The Children's Hospital at Westmead, Sydney, Australia, <sup>8</sup>Department of Radiology, University of Washington, Seattle, WA, United States, <sup>9</sup>Department of Genomic Medicine, Royal Prince Alfred Hospital, Camperdown, Sydney, Australia, <sup>10</sup>Department of Genome Sciences, University of Washington, Seattle, WA, United States, <sup>11</sup>Division of Genetic Medicine, Seattle Children's Hospital, Seattle, WA, United States, <sup>12</sup>Division of Neurology, Children's Hospital of Philadelphia and Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States, <sup>13</sup>School of Genomic Medicine, University of Sydney, Sydney, Australia, <sup>14</sup>Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Pudong, Shanghai, China, <sup>15</sup>Area di Ricerca „Genetica e Malattie Rare”, Ospedale Pediatrico Bambino Gesù - IRCCS, Rome, Italy.

Infantile encephalopathies are a group of clinically and biologically heterogeneous disorders for which the genetic basis remains largely unknown. Here, we report a previously unrecognized syndromic neonatal encephalopathy characterized by profound developmental disability, severe hypotonia, seizures, diminished respiratory drive requiring mechanical ventilation, brain atrophy, corpus callosum dysgenesis, cerebellar vermis hypoplasia, and facial dysmorphism. Biallelic inactivating mutations in TBCK (TBC1 domain-containing kinase) were independently identified by Whole-Exome Sequencing (WES) as the cause of this condition in four unrelated families. Matching these families was facilitated by sharing phenotypic profiles and WES data in a recently released web-based tool (Geno2MP) that links phenotypic information to rare variants in families with Mendelian traits. TBCK is a putative GTPase-activating protein (GAP) for small GTPases of the Rab family and has been shown to control cell growth and proliferation, actin cytoskeleton dynamics, and mTOR signaling. Two of the three mutations are predicted to truncate the protein (c.376C>T [p.Arg126\*] and c.1363A>T [p.Lys455\*]), and loss of the major TBCK isoform was confirmed in primary fibroblasts from one affected individual. The third mutation, p.Arg511His, alters a conserved residue within the TBC1 domain. Structural analysis implicates Arg511 as a required residue for Rab-GAP function, and in silico homology modeling predicts impaired GAP function in the corresponding mutant. These results suggest loss of Rab-GAP activity is the underlying mechanism of disease. In contrast to other disorders caused by dysregulated mTOR signaling associated with focal or global brain overgrowth, impaired TBCK function results in progressive loss of brain volume.

#### P11.079

#### Exome sequencing of rare diseases in founder populations and identification of novel disease associated genes

C. Gonzaga-Jauregui<sup>1</sup>, E. Puffenberger<sup>2</sup>, C. Van Hout<sup>1</sup>, K. Brigatti<sup>2</sup>, A. King<sup>1</sup>, K. Praveen<sup>1</sup>, M. Kuebler<sup>2</sup>, J. G. Reid<sup>1</sup>, J. D. Overton<sup>1</sup>, S. Mellis<sup>1</sup>, A. Shuldiner<sup>1</sup>, F. Dewey<sup>1</sup>, K. Strauss<sup>2</sup>;

<sup>1</sup>Regeneron Genetics Center, Regeneron Pharmaceuticals Inc, Tarrytown, NY, United States, <sup>2</sup>Clinic for Special Children, Strasburg, PA, United States.

Introduction: Founder populations derive from a small number of initial individuals and are often culturally or geographically isolated from gene

inflow from cosmopolitan populations. As a consequence, founder populations maintain a relatively homogeneous genetic background that is amenable for genetic studies and novel gene discovery.

Materials and Methods: We have performed exome sequencing in families within two founder populations of Amish and Mennonite ancestry seen for genetic evaluation at the Clinic for Special Children in Strasburg, PA, USA. We sequenced the exomes of the proband, parents, and all available affected and unaffected siblings. We performed pedigree-based variant analyses and segregation in order to identify candidate disease genes.

Results: As expected for founder populations, we identified known and novel recessive disease genes, including a case of two recessive disorders segregating together in this population. In addition, we have also identified several novel genes responsible for previously unreported rare disorders and novel variants in recently reported intellectual disability and developmental delay genes such as ARID1B, SETBP1, PURA, SYNGAP1, among others.

Conclusions: We demonstrate the utility of genomic sequencing in individuals with genetic disorders and their family members to reach a fast and accurate molecular diagnosis and identify novel disease genes in the context of a relatively homogeneous genetic background provided by founder populations such as the Amish and Mennonites. The spectrum of variation observed ranges from autosomal recessive homozygous variants specific to these populations to sporadic de novo variants, often occurring in genes known to be related to intellectual disability.

#### P11.080

#### Intragenic CASK deletion found in mosaicism in a female patient

J. Sá, R. Lemos, C. Ventura, S. M. Pereira, J. Pinto-Basto, P. Tavares, P. Rendeiro; CGC Genetics, Porto, Portugal.

CASK gene encodes for calcium/calmodulin-dependent serine protein kinase, essential for normal brain development. Disruption of CASK gene is associated with Mental Retardation and Microcephaly with Pontine and Cerebellar Hypoplasia (MICPCH, MIM 300749), where patients present a remarkably consistent phenotype, including severe intellectual disability/developmental delay, severe postnatal microcephaly and a distinctive facial phenotype.

We report a 2-year-old female infant with a 109 Kbp intragenic deletion in CASK gene found in mosaicism, within approximately 25% of the cells. To the best of our knowledge, this is the first case to report mosaicism in a female carrier of intragenic CASK deletion.

Array CGH was performed on an Affymetrix platform, Cytoscan 750K. Data analysis was performed on ChAS Software, Affymetrix (reference NCBI\_hg19). MLPA was performed on peripheral blood following standard protocols.

Patient's clinical report included postnatal microcephaly and reasonable psychomotor development, but slow in the motor area. Neurological examination results were normal. Array CGH revealed a genomic profile with a 109 Kbp deletion at Xp11.4(41,480,031-41,589,514), involving CASK, GPR34 and GPR826 genes. MLPA analysis confirmed a CASK intragenic deletion encompassing exons 4 to 12. Additionally, MLPA also detected a mosaic state of the deletion, in about 25% of the cells, which was not possible to identify on aCGH. Parents were later studied with normal outcome and, therefore, the variant was established as de novo. These results, with high probability, explain the subtle phenotype of moderately slow motor development found the infant.

#### P11.081

#### An infant with a ring chromosome 11 and 11q24 deletion

O. Altıok Clark<sup>1</sup>, B. Nur<sup>2</sup>, B. Karaman<sup>3</sup>, A. Toylu<sup>1</sup>, E. Mihci<sup>2</sup>, S. Berker Karauzum<sup>4</sup>;

<sup>1</sup>Akdeniz University School of Medicine Department of Medical Genetics, Antalya, Turkey,

<sup>2</sup>Akdeniz University School of Medicine Division of Pediatric Genetics, Antalya, Turkey,

<sup>3</sup>Istanbul University School of Medicine Department of Medical Genetics, Istanbul, Turkey,

<sup>4</sup>Akdeniz University School of Medicine Department of Medical Biology and Genetics, Antalya, Turkey.

Ring chromosomes (RCs) are uncommon findings with a frequency of less than 1:30,000, occurring mostly sporadically. Ring formation is caused when both arms of a chromosome break and reunion, leading to the loss of distal segments. Phenotypic abnormalities observed in patients with ring chromosomes may correlate with the deleted regions. Ring chromosome 11 is has only been described in 20 cases in the literature. All of these cases have growth failure and some degree of intellectual disability, in addition to other abnormalities. Herein, we report a 5 month-old infant with 46,XY, r(11) (p15.5?q24?) [47] / 46,XY, dic r(11)(p15.5?q24?) [3] karyotype. FISH analysis with subtelomeric probes for 11p and 11q revealed a terminal deletion on 11q. Additionally, array comparative genomic hybridization refined the



#### P11.079

#### Exome sequencing of rare diseases in founder populations and identification of novel disease associated genes

C. Gonzaga-Jauregui<sup>1</sup>, E. Puffenberger<sup>2</sup>, C. Van Hout<sup>1</sup>, K. Brigatti<sup>2</sup>, A. King<sup>1</sup>, K. Praveen<sup>1</sup>, M. Kuebler<sup>2</sup>, J. G. Reid<sup>1</sup>, J. D. Overton<sup>1</sup>, S. Mellis<sup>1</sup>, A. Shuldiner<sup>1</sup>, F. Dewey<sup>1</sup>, K. Strauss<sup>2</sup>;

<sup>1</sup>Regeneron Genetics Center, Regeneron Pharmaceuticals Inc, Tarrytown, NY, United States, <sup>2</sup>Clinic for Special Children, Strasburg, PA, United States.

Introduction: Founder populations derive from a small number of initial individuals and are often culturally or geographically isolated from gene

deletion to a 8.8Mb region at 11q24.2q25 (126,120,523-134,924,542), with 42 RefSeq genes including disease causing genes such as TIRAP, KIRREL3, ETS1, FLI1, KCNJ1, KCNJ5, BARX2, ST14, NTM, OPCML, JAM3, ACAD8, B3GAT1. The phenotype is significant for thrombocytopenia, which disappeared spontaneously at 6 months, hypospadias, atrial septal defect, and mild motor retardation which has some overlapping features with Jacobsen syndrome that is associated with 11q23 deletion.

To the best of our knowledge, there have only been 21 cases, including our own, of constitutional RC11 described. Herein we discuss the genes that involved with the deletion and phenotypic features associated with Jacobsen Syndrome.

#### P11.082

#### Investigating potential non-Mendelian inheritance patterns in ciliopathies: the case of Joubert Syndrome

I. G. Phelps<sup>1</sup>, M. Grout<sup>1</sup>, J. Dempsey<sup>1</sup>, C. Isabella<sup>1</sup>, D. O'Day<sup>1</sup>, J. Glass<sup>1</sup>, B. O'Roak<sup>2</sup>, J. Shendure<sup>1</sup>, C. center for Mendelian Genomics<sup>3</sup>, D. Doherty<sup>4</sup>, R. Bachmann-Gagescu<sup>4</sup>;

<sup>1</sup>University of Washington-Dept of Pediatrics, Seattle, WA, United States, <sup>2</sup>OHSU-Molecular & Medical Genetics, Portland, OR, United States, <sup>3</sup>University of Washington, Seattle, WA, United States, <sup>4</sup>University of Zurich- Medical Genetics, Zürich, Switzerland.

**Introduction:** Non-mendelian inheritance, "trallelism" and digenicity/oligogenicity (=heterozygous variants in  $\geq 2$  different JS genes), as well as genetic modifiers, have been reported in various ciliopathies. We investigated their possible occurrence in Joubert syndrome (JS), a ciliopathy characterized by a distinctive hindbrain malformation, and typically caused by biallelic mutations in one of  $>25$  genes.

**Methods:** We sequenced 25 JS genes in 386 individuals with JS and 175 controls using targeted capture and next-generation sequencing.

**Results:** Two rare deleterious variants (RDVs) in one JS gene were identified in 248/386 individuals (64%) ("recessively solved patients"). We found no indication of trallelism, since 0/69 unaffected siblings carried the same two RDVs as their affected relative. We identified 24/138 unsolved patients with RDVs in  $\geq 2$  JS genes as candidates for oligogenicity, while 14/175 controls carried RDVs in  $\geq 2$  JS genes. RDV types and patterns differed between unsolved patients and controls. Finally, we investigated whether additional heterozygous RDVs in JS genes, present in 108/248 (43%) solved patients, acted as genetic modifiers. Pair-wise comparisons between samples sharing identical causal RDVs identified no correlation between number of RDVs and disease severity.

**Conclusion:** Our data offer little support for trallelism or oligogenicity as clinically-relevant alternative inheritance mechanisms in JS, and the number of additional RDVs in "solved patients" does not correlate with phenotypic severity. In patients with possible oligogenic inheritance, future work will focus on identifying 2nd RDVs in genes with single RDVs and functional work to assess effects of candidate oligogenic and modifier variants.

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#### P11.083

#### Might the inheritance pattern of a syndrome mislead the clinician? Two different syndromes in a patient

E. Isik<sup>1</sup>, T. Atik<sup>1</sup>, S. E. Keskin<sup>2</sup>, S. Gokben<sup>2</sup>, H. Onay<sup>3</sup>, O. Cogulu<sup>1</sup>, F. Ozkinay<sup>1</sup>;

<sup>1</sup>Ege University Faculty of Medicine, Department of Pediatrics, Subdivision of Pediatric Genetics, Izmir, Turkey, <sup>2</sup>Ege University Faculty of Medicine, Department of Pediatrics, Subdivision of Pediatric Neurology, Izmir, Turkey, Izmir, Turkey, <sup>3</sup>Ege University Faculty of Medicine, Department of Medical Genetics, Izmir, Izmir, Turkey.

**Introduction:** Joubert syndrome (JS) and Cowden syndrome (CS) are two different single gene disorders. JS, a rare autosomal recessive ciliopathy syndrome, is characterised by hypotonia, ataxia, developmental delay and oculomotor apraxia. Nineteen genes responsible for the JS have been identified. CS, an autosomal dominant syndrome, is characterised by macrocephaly, subcutaneous lipomas and susceptibility to cancer development. PTEN gene mutations are responsible for the CS. In this study, we present a patient who shows clinical features of both JS and CS, and carries mutations in both *AHI1* gene and *PTEN* genes.

**Case Report:** A five year-old-boy was referred to pediatric genetics subdivision cause of intellectual disability and epilepsy. He was born to consanguineous parents. On physical examination, his weight and height were below the 3rd percentile and where as his head circumference at the 97th percentile. He had generalised hypotonia, macrocephaly and subcutaneous lipomas. On cranial MRI, cerebellar vermis hypoplasia and molar tooth sign were detected. The diagnosis of JS was established and molecular analysis revealed a homozygous c.2742\_2744delTCT (p.Leu915del) mutation on the *AHI1* gene, on of the JS genes.

Because macrocephaly and subcutaneous lipomas were unusual for JS, an additional genetic disease, CS, was considered in the patient. Sanger sequencing of *PTEN* revealed a heterozygous novel c.381\_385delGGAAA

(p.K128TfsX50) mutation which was inherited from his mother.

**Conclusion:** In the literature, coincidence of these two syndromes have not been previously reported. This case is presented to emphasize blended phenotypes could be resulted from several concomitant single-gene disorders in the same patient.

#### P11.084

#### Kabuki syndrome: a Spanish case series

V. Seidel<sup>1</sup>, S. García-Miñáur<sup>2</sup>, E. Vallespín<sup>2</sup>, V. Martínez-Glez<sup>2</sup>, G. Gordo<sup>2</sup>, A. Naughton<sup>3</sup>, G. Malone<sup>3</sup>, P. Lapunzina<sup>2</sup>, F. Santos-Simarro<sup>2</sup>;

<sup>1</sup>Clinical Genetics/ Pediatrics, Hospital MI Gregorio Marañón, Madrid, Spain, <sup>2</sup>INGEMM-CIBERER, Hospital Universitario La Paz, Madrid, Spain, <sup>3</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom.

**Introduction:** Kabuki syndrome is a clinically heterogeneous multiple congenital malformation syndrome with distinctive facial features and developmental delay. Two histone methyltransferase genes have been identified as causes, KMT2D and KDM6A.

**Material and methods:** We present 20 cases diagnosed with Kabuki syndrome, seen at two large hospitals in Madrid, Spain.

**Results:** Age at diagnosis ranged from 5 months to 16 years. 17 cases had a molecular confirmation (16 KMT2D and 1 KDM6A mutation, all de novo), 2 will be presented in due course and one was negative for both genes. Of the 16 KMT2D mutations 11 were truncating. Molecular details will be provided. The combination of congenital anomalies, developmental delay and characteristic facial features was variable but the distinctive eyebrows and long palpebral fissures reminiscent of the Kabuki make-up of Japanese theatre were present in all. Additional features found in our series, rarely or not previously described as part of the syndrome, were trigonocephaly in one case, unilateral corneal opacity and iris coloboma in another, and autoimmune cholangitis in the girl with KDM6A mutation. One patient died at 10 years from complications of her heart anomalies. Interestingly, one case with a milder phenotype and not so characteristic facial dysmorphism was found to be a mosaic for a KMT2D frameshift mutation c.1275delA (p.Gln425Hisfs\*10) in 30-40% of blood.

**Conclusions:** This is the first series of cases with Kabuki syndrome in Spain. Additional cranial, ophthalmological and hepatic features are shown. A case with mosaicism for Kabuki syndrome is presented.

#### P11.085

#### Under the mask of Kabuki-like syndromes: a pilot Czech study

J. Paderova, J. Drabova, A. Holubova, M. Vlckova, M. Havlovicova, R. Pourova, M.

Malikova, A. Puchmajerova, V. Caganova, A. Krepeleva, M. Macek sr., M. Macek jr.; Department of Biology and Medical Genetics, Charles University Prague - 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic.

**Introduction:** Kabuki (make up) syndrome (KABUK; OMIM 148920) is mainly due to autosomal de novo pathogenic variants. Distinctive facial features and the degree of intellectual disability are broad making its clinical diagnosis difficult even for experienced clinical geneticists, in particular in Kabuki-like phenotypes.

**Material and Methods:** A representative cohort of Czech KABUK / Kabuki-like patients was examined (2013-2015) by Sanger DNA sequencing, MLPA, array CGH, followed by next-generation sequencing-based (NGS) gene panel assays (TruSight One, Illumina). Correlation of molecular genetic- and cytogenetic test outcomes was compared to the reliability of clinical diagnosis. In addition, 3D digital phenotyping of facial gestalt was carried to further characterise these patients.

**Results:** In 24 KABUK / Kabuki-like cases pathogenic variants in *KMT2D* were detected in 9 patients. Array CGH revealed likely pathogenic CNVs on chromosomes X and 15, respectively, in 2 patients. NGS detected likely pathogenic variants in genes associated with intellectual disability (*HUWE1*, *GRIN1*), including genes coding for mandibulo-facial dysostosis (*EFTUD2*, *EDNRA*). Nonetheless, in 5 cases their underlying genetic etiology has not been elucidated, while in the remainder familial consent for further testing is pending.

**Conclusion:** Array CGH and broad NGS-based gene panels have a high diagnostic yield in KABUK/Kabuki-like phenotypes. Genomic testing may foster elucidation of otherwise ambiguous clinical diagnoses. 3D facial gestalt phenotyping proved to be a useful tool for further stratification of such patients and eventual prioritisation of detected variants.

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**P11.086****KAT6A syndrome: expanding the phenotypic spectrum**

P. R. Blackburn, N. J. Boczek, M. A. Cousin, G. J. Beek, E. W. Klee, D. Babovic-Vuksanovic;  
Mayo Clinic, Rochester, MN, United States.

**Introduction:** KAT6A syndrome or autosomal dominant mental retardation 32 (MRD32, OMIM#616268) is a novel disorder caused by mutations in the K(lysine) acetyltransferase 6A (KAT6A) gene, characterized by microcephaly, developmental delay, hypotonia, and intellectual disability. So far, only ten children have been reported. We describe a male patient with the c.3385C>T variant who shares many of the same clinical features as well as arthrogryposis multiplex congenita (AMC), previously undescribed in KAT6A syndrome.

**Materials and Methods:** The patient was born with bilateral cryptorchidism, inguinal hernia, hypotonia and craniofacial dysmorphism. The echocardiogram revealed an atrial septal defect (ASD) and patent ductus arteriosus (PDA). He had contractures of the hands, feet, hips, knees, and elbows. The karyotype and aCGH were normal. Molecular testing for myotonic dystrophy, Prader-Willi syndrome, Rubinstein-Taybi, and Freeman-Sheldon syndrome was negative. Whole exome sequencing revealed a de novo variant in KAT6A.

**Results:** This is the 4th reported case of KAT6A syndrome with the c.3385C>T (p.R1129\*) truncating variant. Given the frequency of this mutation and the fact that it falls within a CpG dinucleotide suggests that it may be prone to spontaneous deamination and likely represents a hotspot mutation in this disorder.

**Conclusions:** Here we present a new case of KAT6A syndrome in a patient with AMC, thus expanding the clinical spectrum of this newly described disorder. Heterozygous truncating mutations in the related KAT6B gene cause genitopatellar syndrome (OMIM#606170) characterized by congenital contractures of the lower extremities and undeveloped patellae, suggesting that KAT6A and KAT6B may have overlapping roles in development.

**P11.087****Diagnosing a rare case of KAT6B related disorder using targeted massively parallel sequencing**

T. Szemes<sup>1,2,3</sup>, J. Radvánszky<sup>1,2</sup>, D. Ďurovčíková<sup>5</sup>, M. Hikkelová<sup>6</sup>, M. Hyblová<sup>3,2</sup>;  
<sup>1</sup>Comenius University Science Park, Bratislava, Slovakia, <sup>2</sup>Geneton Ltd, Bratislava, Slovakia, <sup>3</sup>Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, <sup>4</sup>Institute of Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>5</sup>Department of Medical Genetics, Slovak Medical University, Bratislava, Slovakia, <sup>6</sup>Alpha medical Ltd, Bratislava, Slovakia.

**Introduction:** Say-Barber-Biesecker-Young-Simpson (SBBYS) syndrome and genitopatellar (GTPT) syndrome are clinically similar disorders with certain overlapping symptoms. They are currently considered distinct clinical entities which have been shown to be caused by de novo truncating sequence variants resulting from nonsense and frameshift causing DNA changes in the KAT6B (lysine acetyltransferase 6B) gene.

**Materials and methods:** Family trio with affected child manifesting serious multiple congenital anomalies with unsettled diagnosis was analyzed using multiple method approach including array-CGH and two commercial gene-panel based MPS assays. Identified genetic variants were evaluated using GeneTalk and HGMD software.

**Results:** Among several likely benign genomic variants, we were able to identify a de novo truncating c.4592delA (p.Asn1531Thrs\*18) variant in the last KAT6B exon supporting observed phenotypic features overlapping SBBYS syndrome and GTPT syndrome.

**Conclusion:** We were able to identify a novel genetic variant associated with an overlapping SBBYS syndrome and GTPT syndrome. Our findings also suggest that the clinical distinction between these disorders may be blurred and thus conventional clinical classification can be problematic. Based on our observation we support opinion that disorders associated with KAT6B pathogenic mutations should be referred to as "KAT6B spectrum disorders" or "KAT6B related disorders", rather than their current distinct classification into SBBYS syndrome or GTPT syndrome.

**P11.088****An unusual prenatal presentation of Kleefstra syndrome with congenital diaphragmatic hernia**

S. Giangiobbe<sup>1</sup>, M. F. Bedeschi<sup>1</sup>, F. Ciralli<sup>2</sup>, M. G. Patricelli<sup>3</sup>, C. Cesaretti<sup>1</sup>, L. Ronzon<sup>1</sup>, M. Calvello<sup>1</sup>, F. Lalatta<sup>1</sup>;

<sup>1</sup>Clinical Genetic Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, <sup>2</sup>Neonatal Intensive Care Unit, Department of Clinical Science and Community Health, Università degli Studi di Milano and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>3</sup>Medical Genetic, Molecular Biology and cytogenetics lab, Ospedale San Raffaele, Milan, Italy.

**Introduction:** Kleefstra syndrome (KS) is characterized by intellectual disability, hypotonia and distinctive facial features. It can be caused either by a 9q34.3 deletion that includes the EHMT1 gene or by a EHMT1 mutation. Several additional clinical findings have been observed in KS patients. We describe a case of an unusual prenatal presentation of KS with congenital diaphragmatic hernia (CDH), never reported before as part of the KS phenotypic spectrum.

**Case description:** A 34-year-old woman with unremarkable family and medical history was referred to our department at 21 weeks of gestation of her second pregnancy for genetic counseling. The pregnancy was uneventful until 20 weeks of gestation when morphologic ultrasound detected moderate left CDH, which was then confirmed by a level II scan. CGH analysis on amniocytes revealed a 3,9 Mb de novo deletion on 9q34.2-34.3 that included the EHMT1 gene. A female fetus was delivered vaginally at 35 weeks with a weight of 2100 g and an Apgar score of 9-10. Post-natal echocardiography showed complex cardiac septal and valvular defects. She died a week after from cardiorespiratory failure.

**Conclusions:** The deletion we detected also encompasses the COL5A1 gene, whose loss likely contributed to the development of CDH. CDH may thus be part of the phenotypic spectrum of KS due to deletions that extend over several genes; penetrance seems incomplete as the two cases previously reported in the literature with similar deletions did not develop CDH. As in our case, CDH presence may have a negative prognostic impact on the syndrome.

**P11.089****Kleefstra syndrome diagnosed in an unusual way**

Y. K. Terzi<sup>1</sup>, A. Ozcan<sup>1</sup>, I. Erol<sup>2</sup>, Z. Yilmaz Celik<sup>1</sup>;

<sup>1</sup>Baskent University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Baskent University Faculty of Medicine Department of Pediatric Neurology, Ankara, Turkey.

**Introduction:** Launch of new techniques enabled the diagnosis of genetic disorders more precisely. Every method has its own advantages and disadvantages, thus the diagnostic laboratory uses all methods in an order to make the correct diagnosis. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis is a method used in detection of deletions or duplications of genes. Here we report an 11-year-old boy consulted to our department due to mental retardation and dysmorphic features with a suggested diagnosis of Di George syndrome.

**Materials and methods:** Peripheral blood lymphocyte cultures were set up as well as DNA isolation and following MLPA analysis.

**Results:** The boy was the second child of a 41-year-old mother and father. Conventional cytogenetic analysis revealed a normal karyotype. We did not detect deletion of the Di George region during analyses, however we detected a heterozygous deletion of EHMT1 gene which is used as a reference gene in the commercial kit (P250-B2 DiGeorge Lot No: 0614).

**Conclusions:** MLPA method is a sensitive and handy tool for diagnosis of microdeletion syndromes as well as other conditions that result from genomic imbalances. We diagnosed the patient incidentally while looking for another abnormality. When we re-evaluated the patient after the results, we observed the dysmorphic features were consistent with the diagnosis. Although coincidental findings might be harmful in some circumstances, in this example off-target effect of MLPA help us to make diagnosis.

**P11.090****Lacrimo-auriculo-dento-digital syndrome: Description of two families reveals great phenotypic variability**

A. Beleza-Meireles, E. Hughes, M. Harrison, D. Josifova;  
Guy's Hospital, London, United Kingdom.

Lacrimo-auriculo-dento-digital syndrome (LADD) is an autosomal-dominant condition characterised by abnormalities of the lacrimal and salivary glands, teeth and hands. Only about 60 cases have been described in the literature. Mutations in the FGFR2, FGFR3 and FGF10 genes cause LADD syndrome. We report two familial FGF10 related cases and provide literature review.

Case 1 is a 12 year old girl presenting with dry mouth secondary to salivary glands hypoplasia. She also had absent lacrimal ducts, small ears and dental anomalies. Molecular analysis revealed an exon 3 deletion in FGFR10. Her father, who carried the mutation, had blocked tear ducts as a child. His mother and brother have teeth abnormalities. Her cousin through her paternal uncle was independently diagnosed with LADD syndrome.

Case 2 is an 11 year old girl with complete absence of salivary glands, widespread dental decay, obstructed tear ducts, and cognitive and behavioral difficulties on the autistic spectrum. She developed focal seizures secondary to focal cortical dysplasia identified on brain MRI. Her father underwent several surgical procedures to improve the patency of his nasolacrimal ducts in childhood. He was found to have agenesis of the parotid and right submandibular glands, although he was asymptomatic. Both were found to have the p.Arg78Leu mutation in FGFR10.

This report underlines the phenotypical variability that may be present as a result of FGFR10 mutations, ranging from LADD syndrome to aplasia of the lacrimal and salivary glands (ALSG). ALSG and LADD may represent variable presentations of the same clinical spectrum caused by FGF10 mutations.

#### P11.091

#### Novel likely pathogenic mutation in FGFR2 causes Lacrimo-Auriculo-Dento-Digital syndrome

M. J. Nabais Sá<sup>1,2</sup>, A. M. Fortuna<sup>1,2</sup>, G. Soares<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Centro de Genética Médica Dr. Jacinto de Magalhães / Centro Hospitalar do Porto, Porto, Portugal, <sup>2</sup>Unit for Multidisciplinary Research in Biomedicine, Abel Salazar Institute of Biomedical Sciences, University of Porto, Porto, Portugal.

**Introduction:** Lacrimo-Auriculo-Dento-Digital syndrome (LADD; MIM# 149730) is extremely rare, with only approximately 60 patients reported to date. It is caused by heterozygous mutations in FGF10, FGFR2, or FGFR3, which result in abnormalities of the eyes, ears, teeth and limbs. Phenotypic expressivity is highly variable and genotype-phenotype correlations are not known. Here, we present the clinical and molecular characterization of a female patient with a novel likely pathogenic variant in FGFR2.

**Case Report:** The 2-month-old girl was referred to the Medical Genetics consultation due to bilateral agenesis of the thumbs. She had low-set, simple ears and a cup-shaped right ear. Parents reported alacrimia and absence of lacrimal puncta was observed. Lower central incisors were peg-shaped. Clinical diagnosis of LADD syndrome prompted direct sequencing of FGF10, FGFR2 and FGFR3. A novel, de novo heterozygous missense variant was identified in FGFR2 (NM\_000141.4): c.1928C>A, p.(Ala643Glu). Ala643 is conserved down to fruitfly and is in the tyrosine kinase domain of the fibroblast growth factor receptor. Bioinformatic prediction tools supported potential pathogenicity of the change. We propose that p.(Ala643Glu) reduces the receptor activity via a dominant-negative effect.

**Conclusion:** This clinical case expands the spectrum of genetic variants that cause LADD syndrome. The confirmation of the clinical diagnosis enabled precise genetic counselling to the parents. Given a low recurrence risk, in the subsequent pregnancy, they opted to do prenatal diagnosis by ultrasound to exclude major malformations associated with LADD syndrome.

#### P11.092

#### Leri's pleonosteosis associated with a duplication of 8q22.1, a case report from Argentina

S. Menazzi<sup>1</sup>, A. P. Solari<sup>1</sup>, L. Espeche<sup>1</sup>, M. A. Mori<sup>2</sup>, J. Nevado<sup>2</sup>, S. Rozental<sup>1</sup>;

<sup>1</sup>National Medical Genetics Centre, Buenos Aires, Argentina, <sup>2</sup>INGEMM-La Paz University Hospital, Madrid, Spain.

Leri's pleonosteosis is an autosomal dominant congenital rheumatic disease associated with digital anomalies (including contractures and limited motion), facial dysmorphism, chronic pain, short stature, spinal nerve compression and scleroderma-like skin. This entity has been reported in a family with a microduplication of 8q22.1 [Banka et al].

We describe a 36-year-old woman with a duplication of 8q22.1 with facial dysmorphism (short palpebral fissures, hypoplasia of the ala nasi), brachydactyly, growth in 3rd centiles, mild intellectual disability and digital and vertebral anomalies. She did not finish primary school and has a non-specified behavior disorder, and was reluctant to being interviewed or examined. She had 3 healthy siblings with normal phenotype, her parents were non-consanguineous and her mother had similar dysmorphic features. The patient had recently been diagnosed with hypothyroidism, had conductive hearing loss (due to tympanic perforation), a deviated nasal septum and a lumbosacral transitional vertebrae with disc protrusion at L3-L4. Her hands and feet X-ray revealed short and broad phalanges and metacarpals. Karyotype analysis with G-banding and MLPA (SALSA P036 and P070) were

performed, with normal results, and Karyoarray® (Agilent 60K) revealed a 1 Mb duplication of 8q22.1, involving genes GDF6 and SDC2. To the best of our knowledge this is the second case report of the association between Leri's pleonosteosis and duplication of 8q22.1, expanding the clinical spectrum of this disease, and supports the putative genetic basis of this condition. Specialists in these fields (rheumatology and genetics) should be aware of this disease for proper assessment and genetic counseling.

#### P11.093

#### A de novo missense mutation in LMNA associated with a segmental progeroid phenotype in a Turkish patient

M. C. Ergoren<sup>1</sup>, S. G. Temel<sup>2,3</sup>, F. Uysal<sup>4</sup>, M. Schotik<sup>5</sup>, J. Altmüller<sup>6</sup>, P. Nürnberg<sup>6</sup>, G. Yigit<sup>7</sup>, B. Wolnik<sup>8</sup>, E. Cilt<sup>9</sup>;

<sup>1</sup>Near East University, Faculty of Medicine, Department of Medical Biology, Nicosia, Cyprus, <sup>2</sup>Near East University, Faculty of Medicine, Department of Histology & Embryology, Nicosia, Cyprus, <sup>3</sup>Uludag University, Faculty of Medicine, Department of Histology & Embryology, Bursa, Turkey, <sup>4</sup>Uludag University, Faculty of Medicine, Department of Pediatric Cardiology, Bursa, Turkey, <sup>5</sup>University of Cologne, Institute for Developmental Biology, Cologne, Germany, <sup>6</sup>University of Cologne, Cologne Center for Genomics, Cologne, Germany, <sup>7</sup>University Medical Center Göttingen, Institute of Human Genetics, University, Göttingen, Germany.

Mutations in the LMNA gene encoding lamin A/C are associated with a wide spectrum of phenotypes affecting different tissues and organ systems. The clinical features of these disorders can overlap, however are generally categorized into two groups: dilated cardiomyopathy, neuromuscular disorders; and premature aging, lipodystrophy disorders. The Hutchinson-Gilford progeria syndrome and mandibuloacral dysplasia are segmental progeroid syndromes caused by mutations within LMNA. Dominant LMNA mutations have also been reported in patients with a less well-characterized so called atypical progeroid syndrome.

We report a 15 years old female who initially presented with progeroid features, growth retardation and facial dysmorphism including prominent eyes, full cheeks, marked retrognathia, high arched palate, beaked nose, scleroderma atrophic skin, and sparse thin scalp hair. Additionally, she presented with limitation of extension of both wrists and elbows, and slight scoliosis. Physical examination revealed low weight and short stature and although she has regular menstruation cycles she has poor breast development. Her ECG showed a structurally normal heart, with normal left ventricle diameter and ejection fraction and no sign for dilated cardiomyopathy, but severe tricuspid regurgitation and mild mitral regurgitation. Trio exome sequencing revealed the presence of a de novo heterozygous missense mutation, c.176T>G resulting in a leucine to arginine change at codon 59. Three female patients have been described with a LMNA mutation affecting leucine at position 59; interestingly however, our patient does not show primary ovarian failure and dilated cardiomyopathy as described in the other patients, indicating variable expression of phenotypic presentation.

#### P11.094

#### Perinatal presentation of Loeys-Dietz Syndrome due to a novel mutation in TGFRB2. Further delineation of the neonatal phenotype

I. Valenzuela<sup>1</sup>, P. Fernandez<sup>1</sup>, F. Munell<sup>2</sup>, A. Sanchez<sup>3</sup>, G. Giralt<sup>4</sup>, A. Linde<sup>5</sup>, T. Vendrell<sup>1</sup>, E. Tizzano<sup>6</sup>;

<sup>1</sup>Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron Barcelona, Spain, <sup>2</sup>Department of Neuropediatrics, Hospital Vall d'Hebron, Barcelona, Spain, <sup>3</sup>Department of Neuroradiology, Hospital Vall d'Hebron Barcelona, Spain, <sup>4</sup>Department of cardiology, Hospital Vall d'Hebron Barcelona, Spain, <sup>5</sup>Department of Neonatology, Hospital Vall d'Hebron Barcelona, Spain, <sup>6</sup>Barcelona, Spain.

Loeys-Dietz syndrome (LDS) is an autosomal dominant connective tissue disorder characterized mainly by cardiovascular, craniofacial and skeletal features.

We report on a patient whose prenatal examination implied the diagnosis of arthrogryposis multiplex congenita and neonatal assessment showed craniofacial and cardiovascular findings that suggested the diagnosis of LDS. The clinical diagnosis was confirmed by detection of a de novo mutation in the TGFRB2 gene c.1381T>C (p.Cys461Arg) previously unreported in databases and predicted to be pathological and deleterious using five in silico predictive programs.

Few prenatal and neonatal cases of LDS have been reported in the literature. Our patient presented aortic root dilatation, arterial tortuosity and musculoskeletal anomalies. Remarkable craniofacial defects include hypertelorism, blue sclerae, strabismus, bifid uvula and cleft palate. Two interesting findings previously underreported were facial milia and multiple central nervous system cysts mainly in the anterior horns of the lateral ventricles. We also review the clinical manifestations to delineate a recognizable phe-

notype at this stage. In the published cases with neonatal presentation, aortic root dilatation was the first sign in 30% of patients. In the remaining cases, (70%), muscle and skeletal findings were the initial manifestation. This suggests that when arthrogryposis multiplex congenita is detected in a foetus or in a neonate the differential diagnosis should include the possibility of LDS even though no cardiologic finding at diagnosis.

It is important to delineate the clinical manifestations of neonatal LDS to allow a prompt diagnosis and subsequent management to prevent detrimental complications.

## P11.095

### Delination of a Marker Chromosome by aCGH in a Patient with Undiagnosed Intellectual Disability and Neurodevelopmental Disorder

R. Celik<sup>1</sup>, I. Suer<sup>2</sup>, A. Koparır<sup>2</sup>, A. Onul<sup>1,3</sup>, A. Yüksel<sup>1</sup>, M. Ozan<sup>3,5,6</sup>,

<sup>1</sup>Biruni University, Reyhan Genome Center, Istanbul, Turkey, <sup>2</sup>Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Turkey, <sup>3</sup>Biruni University, Department of Medical Genetics/Molecular Biology and Genetics, Istanbul, Turkey, <sup>4</sup>Biruni University, Istanbul, Turkey, <sup>5</sup>Department of Medical Genetics, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey, <sup>6</sup>Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Complex small supernumerary marker chromosomes (sSMC) are one of the subgroups of sSMC and consist of more than one chromosome. Identifying the origin of marker chromosome is very important for clinicians to better manage the patients.

**Material and Methods:** A 32 month-old girl admitted to our center for development disorder and mental/motor disabilities. Conventional cytogenetic technique was used for cytogenetic analysis. aCGH analysis applied by Illumina Human CytoSNP 12 Beadchip.

**Results:** Patient's clinical features include microcephaly, distinct eye structure, broad nasal root, micrognathia, highly arched palate, and small extremities. Cytogenetic analysis revealed a *de novo* marker chromosome with an unknown origin. SNP array analysis determined by Illumina Human CytoSNP 12 Beadchip showed two duplications: 12.7 Mb duplication covering 15q11.1-q13.3 and 542 Kb covering 22q11.23-q12.1. According to the Decipher database 15q11.1-q13.3 duplication was reported to be associated with hypotony, deep philtrum, highly arched palate, intellectual disability, hypothalamo-hypophyseal tract abnormalities, seizures and optic atrophy. On the other hand the phenotypic features of 22q11.23-q12.1 were reported as abnormal face and neonatal hypotony.

**Conclusion:** In conclusion, we were able to determine the source of marker chromosome previously undetermined. We propose a targeted array analysis to uncover marker chromosomes.

## P11.096

### Additional findings in the Matthew-Wood syndrome

P. Ozyavuz Cubuk<sup>1</sup>, L. Ho<sup>2</sup>, B. Reversade<sup>2,3</sup>, E. F. Percin<sup>1</sup>,

<sup>1</sup>Gazi University Hospital, Medical Genetics Department, Ankara, Turkey, <sup>2</sup>Institute of Medical Biology, Human Embryology Laboratory, A\*STAR, Singapore, Singapore, <sup>3</sup>Department of Paediatrics, National University of Singapore, Singapore, Singapore.

**Introduction:** Matthew-Wood syndrome (MWS), also termed microphthalmia, syndromic 9 (MCOPS9, MIM 601186), is an autosomal recessive disorder characterised by ocular, respiratory and cardiac abnormalities. Mutations in retinoic acid 6 gene (STRA6) have been reported in clinically diagnosed patients with MWS. Here we presented a case with MWS, who has characteristic findings of the syndrome as well as dextrocardia and bilateral streak gonads which was not defined previously in this syndrome.

**Case:** The proband was a stillborn girl baby with normal intrauterine growth. Postmortem examination and autopsy showed that bilateral anophthalmia, bilateral pulmonary agenesis, complete tracheoesophageal fistula, complex cardiac defects, dextrocardia, malrotation of the colon, right cystic renal dysplasia and bilateral streak gonads.

**Results:** Molecular analysis showed a homozygous exonic missense C>T mutation in STRA6 gene (NM\_022369) at coding position 878 (c.878C>T, p.Pro293Leu, rs118203958).

**Discussion:** It is known that dextrocardia and streak gonads are undefined in patients with MWS to date, but they are a part of PAGOD syndrome which demonstrates significant phenotypic overlap with MWS. The etiology of PAGOD syndrome is unknown but hypothesis that a vitamin A metabolic defect. This situation suggests that these two new findings completely arise as a result of vitamin A deficiency. It should be made the molecular tests in similar cases because of it will not allow to be made differential diagnosis with only clinical diagnosis.

## P11.097

### A case with Meckel-Gruber syndrome (dysencephalia splanchnocystica) caused by a novel mutation in CEP290 gene.

A. Bakar, V. Topcu, E. S. Çakar;

Zekai Tahir Burak Education and Research Hospital, Ankara, Turkey.

Meckel-Gruber syndrome (MKS) is a rare pleiotropic autosomal recessive developmental disorder which was first described by Johann Friedrich Meckel in 1822 and G.B. Gruber in 1934. More than 200 cases have been reported worldwide with an incidence ranging from 1:13.250 to 1:140.000. It's characterized with typical manifestations of occipital encephalocele, bilateral polycystic kidneys and post-axial polydactyly. Here, we report a foetus, product of a consanguineous marriage who was demonstrated to have oligohydramnios, encephalocele and multicystic kidney detected by USG in antenatal assessment at the 17th week of gestation. The foetus was diagnosed Meckel-Gruber syndrome (MKS). Parents approved termination of pregnancy due to multiple foetal anomalies. A skin biopsy was taken during postabortal examination. Molecular genetic analysis of fetal DNA using NGS revealed a novel mutation in homozygous condition, c.1915G>T (p.Glu639\*), in exon 20 of CEP290. DNA analysis from peripheral lymphocytes of parents showed the same mutation in heterozygous condition.

## P11.099

### Meier-Gorlin syndrome: GMNN mutations causing autosomal-dominant primordial dwarfism compared to pre-replication complex gene mutations causing autosomal-recessive primordial dwarfism

E. M. H. F. Bongers<sup>1</sup>, S. A. de Munnik<sup>1</sup>, J. Schoots<sup>1</sup>, L. C. Burridge<sup>2,3</sup>, S. R. Lalani<sup>2</sup>, W. Charng<sup>2</sup>, J. R. Lupski<sup>2,3,4</sup>, H. G. Brunner<sup>1</sup>, D. Lugtenberg<sup>1</sup>, Y. Yang<sup>2,5</sup>,

<sup>1</sup>Dept of Human Genetics, Radboud university medical center, Nijmegen, Netherlands,

<sup>2</sup>Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States,

<sup>3</sup>Dept of Pediatrics, Texas Children's Hospital, Houston, TX, United States,

<sup>4</sup>Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, United States,

<sup>5</sup>Exome Laboratory, Baylor Miraca Genetics Laboratories, Houston, TX, United States.

Meier-Gorlin syndrome (MGS) is a genetically heterogeneous microcephalic primordial dwarfism syndrome known to be caused by biallelic loss-of-function mutations in one of five genes encoding proteins involved in DNA replication initiation (ORC1 [origin recognition complex 1]/ORC4/ORC6/ CDT1 and CDC6). Thus far, mutations in these genes with an autosomal-recessive inheritance pattern have been established in about 80% of individuals with primary clinical hallmarks of MGS, including short stature, microtia and small/absent patellae. Recently, we identified *de novo* heterozygous (two truncating and one missense) mutations in GMNN by whole-exome sequencing, encoding the DNA replication inhibitor geminin in three individuals fulfilling the clinical diagnostic criteria of MGS. A gain-of-function mechanism was shown to result in autosomal-dominant MGS. The three individuals with *de novo* mutations in GMNN demonstrate a more severe growth retardation compared to 38 individuals with pre-replication complex gene mutations, fullness of the peri-orbital region, lumbar hyperlordosis and developmental delay and/or cognitive impairment, a rare finding in autosomal-recessive MGS. Based on the severity of the phenotype, we speculate that ORC1 and GMNN mutations might perturb DNA replication to a greater extent than the other MGS-associated genes, and perhaps GMNN mutations have a more profound effect on the replicative burst and cell growth required for brain development, head size expansion, and height. The identification of autosomal-dominant MGS caused by GMNN mutations expands the genetic heterogeneity of MGS, helps identifying the molecular etiology in additional individuals, and provides further insights into the relationship between DNA replication, cell growth, and organismal development.

## P11.100

### Exome sequencing diagnosed a family with two rare recessive diseases

D. Svaneby, M. M. Aagaard, A. B. Bojesen;

Clinical Genetics Department, Vejle, Denmark.

A non-consanguineous family had four children, two severely affected sons, a healthy son and daughter. The phenotypic characteristics of the affected sons are; severe hypertrophic cardiomyopathy, micro-lissencephaly, seizures, growth retardation, and skin affection. Previous genetic investigation revealed a MYH7B mutation in the first affected son, but no explanation for the brain malformation was found. Despite normal prenatal ultrasound and MRI scans, the second affected son was born. Exome sequencing was then performed to reveal the genetic cause for the micro-lissencephaly.

**Material and Methods:** We performed exome sequencing on the parents and the affected sons. We analyzed data according to inheritance pattern and phenotype defined by HPO terms.

**Results:** The two affected sons harbor mutations in two genes, both in compound heterozygotic form. Two mutations in MYH7B, a frameshift deletion and a non-sense mutation (chr20:g.33567201C>T and chr20:g.33586952ACAG>-) and two mutations in RTTN, a splice-site and a missense mutation (chr18:g.67813014C>T and chr18:g.67833426T>G). Pathogenic MYH7B mutations have been associated with hypertrophic cardiomyopathy, while expected pathogenic RTTN mutations affecting both alleles have recently been associated with growth retardation and a severe brain phenotype with microcephaly.

**Conclusion:** We diagnosed two different recessive diseases in two severely affected brothers with a complex phenotype using exome sequencing. In cases with a well-defined phenotype and inheritance but no obvious matching syndrome, two diseases are to be considered. Together, the two recessive diseases can explain the phenotype in this family, highlighting the strength of parents-offspring exome sequencing to diagnose two rare recessive diseases in the same family.

#### P11.102

#### Microcephalic osteodysplastic primordial dwarfism type II (MOPDII) with 46,XY complete gonadal dysgenesis: co-occurrence of two rare condition in a single patient

A. Gezdirici<sup>1</sup>, T. Akcay<sup>2</sup>, A. Jackson<sup>3</sup>;

<sup>1</sup>Department of Medical Genetics, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkey, <sup>2</sup>Department of Pediatric Endocrinology, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkey, <sup>3</sup>MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom.

**Background:** Microcephalic osteodysplastic primordial dwarfism type II (MOPDII) is a form of microcephalic primordial dwarfism characterized by extreme pre and postnatal growth retardation, severe microcephaly, skeletal dysplasia, abnormal dentition, insulin resistance, and increased risk for cerebrovascular disease. MOPD II is caused by mutations in PCNT, encoding pericentrin, which anchors a wide range of centrosomal proteins and proteins in complexes during cell division.

46,XY complete gonadal dysgenesis (46,XY CGD) is characterized by a 46,XY karyotype, normal female external genitalia, completely undeveloped (streak) gonads, no sperm production, and presence of normal mullerian structures.

**Methods and Results:** Here, we report a 12 years old female patient who has severe pre and postnatal growth retardation, severe microcephaly, retrognathia, relatively large nose, high-pitched voice, skin hyperpigmentation, left hip dysplasia, clitoral hypertrophy with diagnosis of MOPDII. Mutational screening of the PCNT gene showed c.3465-1G>A homozygous splice acceptor site mutation. This mutation is highly likely pathogenic variant but which is not described in the literature so far. Interestingly, she has 46,XY karyotype and SRY FISH was found positive. Abdominal MRI revealed hypoplastic uterus and ovaries. We thought that the patient has second genetic etiology for this situation. Whole exom sequencing (WES) is ongoing.

**Conclusion:** To our best knowledge, this is the first report with combined phenotypes of MOPDII and 46,XY CGD. We present this case because of the coexistence of two rare condition in the same patient.

#### P11.103

#### Identification of a heterozygous BUB1B mutation in a family with mosaic variegated aneuploidy syndrome

G. Kayhan, M. A. Ergun, F. E. Percin;

Medical Genetics Department, Gazi University Hospital, Ankara, Turkey.

**Introduction:** Mosaic variegated aneuploidy syndrome (MVA) 1 is caused by biallelic mutations in the BUB1B gene and characterized by severe microcephaly, developmental delay, increased risk of malignancy and mosaic aneuploidies involving multiple different chromosomes. Also, heterozygous mutations of BUB1B gene cause premature chromatid separation (PCS) trait. Opposite of this classical knowledge, heterozygosity for mutations in the BUB1B gene has been previously identified in 7 unrelated MVA families. Here we present a new MVA family with heterozygous mutation in the BUB1B gene.

**Case:** The proband was 11-year-old male with severe microcephaly, intellectual disability and Eisenmenger's syndrome. Two siblings with the same findings had died.

**Results:** Whole exome sequencing identified heterozygous c.C580T (p.R194X) mutation (maternal) in the BUB1B gene. Karyotype analysis showed various aneuploidies in 25% of his cells. The proband, mother and father showed 55%, 35%, 0% PCS cells in the lymphocyte culture, respectively. There was no mutation in the CEP57 gene related with MVA2.

**Conclusions:** The difference of PCS cells ratio of the proband (55%) and the unaffected mother (35%) shows that there is a relationship between PCS cell ratio and MVA phenotype. PCS cells are generally greater than 50% in MVA patients. Although, authors have focused on that the second allele haplotype 6G3 (26020GT, 1046GA, D15S994) may be associated with the MVA phenotype, G1046A (paternal) polymorphism was determined in the second allele in our proband. So, further studies are needed to clarify the relationship between the BUB1B mutations and PCS/MVA phenotypes.

#### P11.104

#### Mosaic 19q12q13.11 deletion associated with mild intellectual disability and congenital malformations

O. S. Kurinnaia<sup>1,2,3</sup>, S. G. Vorsanova<sup>1,2,3</sup>, M. A. Zelenova<sup>2,1,3</sup>, K. S. Vasin<sup>2,1,3</sup>, Y. B. Yurov<sup>2,1,3</sup>, I. Y. Iourov<sup>2,1,4</sup>;

<sup>1</sup>Research and Clinical Institute for Pediatrics named after Y.E Veltishev at the Pirogov Russian National Research Medical University, Ministry of Health, Moscow, Russian Federation, <sup>2</sup>Mental Health Research Center, Moscow, Russian Federation, <sup>3</sup>Moscow State University of Psychology and Education, Moscow, Russian Federation, <sup>4</sup>Department of Medical Genetics, Russian Medical Academy of Postgraduate Education, Ministry of Health, Moscow, Russian Federation.

**Introduction:** Since chromosome 19 is one of the most gene-rich in a human genome, most of the rearrangements are severely deleterious or incompatible with life. For instance, chromosome 19q13.11 microdeletion syndrome is generally associated with intellectual disability, speech disturbances, pre- and post-natal growth retardation, microcephaly, ectodermal dysplasia, and male genital malformations. Here, we describe a similar mosaic case.

**Materials and Methods.** Cytogenetic and molecular karyotyping with bioinformatic analysis were used to address the case of an 8-year-old boy with mild intellectual disability, ADHD, epilepsy, congenital malformations (i.e. pectus excavatum, hydrocele and inguinal hernia, flatfoot, sinus node dysfunction) and facial dysmorphisms (low-set dysplastic auricles, almond-shaped eyes with downslanting palpebral fissures).

**Results.** A mosaic 19q12q13.11 deletion (29,373,242-34,341,260) was detected in about 70% of the cells (encompassing almost 5 Mb and 20 OMIM genes). Using bioinformatic analysis, we were able to define candidate genes for the index case: *UQCRCFS1* (involved in metabolic pathways and neurodegenerative diseases pathways), *CCNE1* (involved in cell cycle, p53 signaling and PI3K-Akt signaling pathways) and *KCTD15* (a negative regulator of AP2-alpha regulating neural crest formation during early development).

**Conclusions.** Despite the deletion at the 19q13.11 deletion syndrome region, neither the critical region nor most of the described cases have matched the deletion described. Additionally, since there have been no descriptions on similar mosaic deletions, we concluded that this is the first case of mosaic 19q12q13.11 deletion that lead to an atypical neuropsychiatric phenotype. Supported by the Russian Science Foundation (grant: 14-15-00411).

#### P11.105

#### 45,X/47,XX,+18 mosaicism in a young girl: clinical presentation and different distribution of cell lines in peripheral blood lymphocytes and skin fibroblasts

P. Battaglia<sup>1</sup>, A. Capucci<sup>1</sup>, I. Loddo<sup>1</sup>, A. Tanzariello<sup>1</sup>, L. Serra<sup>2</sup>, M. Lanari<sup>2</sup>, O. Calabrese<sup>1</sup>;

<sup>1</sup>UOC Genetica Medica - AUSL Imola, Imola, Italy, <sup>2</sup>UOC Pediatria - AUSL Imola, Imola, Italy.

The female proband, the third child of healthy, unrelated parents, was born at term after an uneventful pregnancy, except mild IUGR during the last month. Birth weight was 2530g, length 45cm (<3°P). Psychomotor development was normal, with mild language delay (first words at 3 years). Menarche occurred at 11 years and 5 months, characterized by menorrhagia. She was referred for clinical and cytogenetic evaluation at the age of 14 because of short stature and anemia.

On clinical examination, height was 135,5 m (<<3°P), weight 50,5kg (50-75°P), occipitofrontal circumference 53cm (50°P).

She presented round face, short neck, thin upper lip, short philtrum, narrow palate, short hands, several nevi on face and trunk, left fourth metatarsal shortening, scoliosis and mild myopia.

Cytogenetic investigation performed on peripheral blood lymphocytes showed the presence of a mosaicism 45,X/47,XX,+18 characterized by two cell lines (85% trisomy 18 and 15% monosomy X), confirmed by FISH analysis. The distribution of cell lines was found to be reversed in skin fibroblasts, where FISH analysis with pericentromeric specific probes showed the presence of three cell lines: monosomy X and trisomy 18 cell lines in 78,3% and 19,6% respectively, and a female normal cell lines in 2,1%.

Constitutional mosaicism for two distinct chromosome aneuploidies is a rare cytogenetic abnormality. Clinical manifestations depend on the distribution of the aneuploid cell lines in different tissues. In our proband, the

prevalence of monosomy X cell lines and the presence of a normal cell line in skin fibroblasts, better correlates with the mild clinical picture.

## P11.106

### Case report: Tandem duplication in ZEB2 in a patient with suspected Mowat-Wilson syndrome

**K. Wittkowski**, A. Munzig, M. Ziegler, S. Chahrokh-Zadeh, D. Wahl, I. Rost; Center for Human Genetics and Laboratory Diagnostics (AHC), 82152 Martinsried, Germany.

Mowat-Wilson syndrome is a multiple congenital anomaly syndrome mainly characterized by typical dysmorphic features, severe intellectual disability, microcephaly and Hirschsprung disease. In addition, organic anomalies and seizures are common. Mutations of the ZEB2 gene were identified as disease-causing and haploinsufficiency of ZEB2 has been reported to cause Mowat-Wilson syndrome. The ZEB2 gene encodes a transcription factor which is involved in the development of the neural crest and derived structures. We report on a 3-year old boy, born to healthy parents, presenting with a complex valvular heart defect, short stature, microcephaly, craniofacial dysmorphia, absent speech and delayed motor development. Initial genetic investigations of Angelman-syndrome (methylation assay, deletion-/UPD-testing, sequencing of *UBE3A* gene) and Pitt-Hopkins syndrome (sequencing and MLPA analysis of *TCF4* gene) revealed negative results.

We performed molecular genetic diagnostics of the ZEB2 gene consisting of Sanger sequencing and MLPA (multiplex ligation-dependent probe amplification) analysis. By MLPA analysis we identified a heterozygous duplication of exon 9. Quantitative PCR confirmed this single exon duplication. Analysis of both parents revealed that the deletion had occurred de novo in the patient. By sequencing an RT-PCR fragment we could demonstrate on RNA level that both copies of exon 9 are arranged in tandem. This duplication results in a translational frame shift and a premature translational termination signal. It has not yet been described in literature and databases. As the tandem duplication of exon 9 of ZEB2 probably leads to loss of function of one allele it is likely to cause the clinical appearance of the patient.

## P11.107

### PIG-o-pathies, a new cause of mental retardation, hypotonia, seizures and congenital anomalies

**E. Zonneveld-Huijssoon**, P. M. van Hasselt, A. Bloem, L. de Vries, M. van Haelst, K. Lichtenbelt; UMC Utrecht, Utrecht, Netherlands.

**Introduction:** Whole exome sequencing reveals increasing numbers of new disease genes. Genes of the GPI-bioanchor synthesis-pathway are being reported as new causes for a number of syndromes. At this moment, at least 10 genes of this pathway are reported in OMIM with a known phenotype. **Methods:** We recently diagnosed three patients with a PIG-o-pathy (PIGA, PIGV and PIGN) and applied an immunological assay for the expression of GPI-coupled proteins, which allows for functional testing of variants of unknown significance in GPI-bioanchor genes. Additionally, we made an overview of all clinical features that have thus far been reported in the literature in PIG-o-pathy patients and categorized them based on occurrence and pivotalness for the diagnosis.

**Results:** The expression of GPI-coupled proteins was reduced in our patient with two PIGN variants of unknown significance. Moreover, this patient showed persistent restricted diffusion in structures in the mesencephalon and diencephalon on brain MRI till 15 months of age. We here show that common features like severe developmental delay, hypotonia, seizures and more distinguishing pivotal features like elevated alkaline phosphatase, vesicourethral reflux, hypoplastic nails and anorectal anomalies are associated with PIG-o-pathies.

**Conclusion:** An immunological assay can be useful in the interpretation of variants of unknown significance in genes of the GPI-bioanchor synthesis pathway. Moreover, as the common features in PIG-o-pathies are aspecific, the more pivotal features reported here may further help in establishing the diagnosis. Whether the observed brain MRI changes are specific for PIG-o-pathies remains to be elucidated.

## P11.108

### Nucleotide excision repair diseases : from Nobel prize 2015 to the unraveling of uncommon and incomplete patient phenotypes

**N. Calmels<sup>1</sup>, M. Spitz<sup>2</sup>, G. Greff<sup>2</sup>, N. Kempf<sup>3</sup>, S. Samimi<sup>4</sup>, C. Obringer<sup>5</sup>, J. Tarabeux<sup>4</sup>, M. Miguet<sup>1</sup>, L. Mary<sup>1</sup>, D. Bessis<sup>4</sup>, J. Gardeazabal<sup>5</sup>, B. Gener<sup>6</sup>, I. Llano-Rivas<sup>6</sup>, A. Mazur<sup>7</sup>, B. Keren<sup>8,9</sup>, C. Depienne<sup>8,9</sup>, J. Muller<sup>1,3</sup>, J. Mandel<sup>1</sup>, V. Laugel<sup>1,2</sup>**

<sup>1</sup>Laboratoire de Diagnostic Génétique, Hopitaux Universitaires de Strasbourg, Institut de Génétique Médicale d'Alsace, Strasbourg, France, <sup>2</sup>Service de Pédiatrie, Hopitaux Universitaires de Strasbourg, Strasbourg, France, <sup>3</sup>Laboratoire de Génétique Médicale-INSERM U1112, Institut de Génétique Médicale d'Alsace, Faculté de médecine de Strasbourg, Strasbourg, France, <sup>4</sup>Département de Dermatologie, Hôpital Saint-Eloi, Montpellier, France, <sup>5</sup>Servicio de Dermatología. Cruces University Hospital. BioCruces Health Research Institute, Bizkaia, Spain, <sup>6</sup>Servicio de Genética. Cruces University Hospital, BioCruces Health Research Institute, Bizkaia, Spain, <sup>7</sup>Department of Pediatrics, Pediatric Endocrinology and Diabetes, Faculty of Medicine, University of, Rzeszow, Rzeszow, Poland, <sup>8</sup>AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, Paris, France, <sup>9</sup>Sorbonne Universités, UPMC Univ Paris 06, Inserm, CNRS, UMR 7225, ICM, Paris, France.

Compromised nucleotide excision repair (NER) activity (deciphered by Aziz Sancar, 2015 Nobel laureate) causes various autosomal recessive diseases including the cancer-prone disorder *xeroderma pigmentosum* (XP) and the severe multisystem condition, Cockayne syndrome (CS). Clinical recognition and molecular characterization of these defects remain challenging, due to overlapping symptoms and genetic heterogeneity. Our goal was to develop a single mutation-screening strategy for these diseases.

We set up a NGS assay based on the enrichment of 16 NER-related genes by multiplex amplification coupled with sequencing on PGM(Ion Torrent). We prospectively analyzed the samples from an international cohort of 40 patients referred for suspected NER-related disorder and we compared the corresponding phenotypes to our personal cohort of 126 NER patients previously characterized by classical sequencing.

We identified causative mutations in 43% of cases, including patients with very mild, incomplete or combined phenotypes and uncommon genotypes (Table1). Four unrelated XP patients from the Basque country revealed a common splicing mutation in *POLH* demonstrating a new founder effect. We also identified two patients linked to the very rarely implicated genes *ERCC3(XPB)* and *ERCC5(XPG)*.

Targeted NGS is efficient for the molecular diagnosis of NER-related disorders. It is particularly useful for phenotypes with combined features or unusually mild symptoms. Molecular characterization allowed appropriate clinical follow-up of the patients, and helped refining the clinical spectrum of NER diseases. Granted from Agence de Biomedecine.

HGNC gene name (legacy name)	Number of patients	Final diagnosis	New mutations discovered in this study	Distinctive characteristic
ERCC8(CSA)	5	Cockayne syndrome	6	2 patients with unusual mild clinical presentation
ERCC6(CSB)	4	Cockayne syndrome	2	
POLH	4	Xeroderma pigmentosum	2	Founder effect in Northern Spain
ERCC2(XPD)	2	Xeroderma pigmentosum		Refining the clinical follow-up
ERCC3(XPB)	1	Xeroderma pigmentosum UV-sensitive syndrome	1	Mild clinical presentation. Only 9 patients described worldwide
ERCC5(XPG)	1	XP/CS complex	1, the first splicing mutation	Only 20 patients described worldwide

## P11.109

### Diagnostics of neurofibromatosis - atypical cases

**J. Štíka<sup>1</sup>, V. Hořnová<sup>2</sup>**

<sup>1</sup>synlab genetics s.r.o., Prague, Czech Republic, <sup>2</sup>Hospital Jihlava, Jihlava, Czech Republic.

**Introduction:** The occurrence of cafe-au-lait spots (CALS) in the skin is associated with a number of severe clinical syndromes. Among them, the most common is neurofibromatosis type 1 (NF), caused by mutations in the *NF1* gene.

**Materials and Methods:** Two unrelated children, female and male, with multiple CALS but without further clinical signs of NF were examined. The same clinical phenotype was also found in other members of the two families. In these individuals, a mutation analysis of the *NF1* gene based on amplicon next generation sequencing (GS-Junior, Roche) with primers amplifying all exons and exon-intron boundaries was performed. Ligated adaptors were used for library preparation.

**Results:** Two different mutations were found in the two families. In the female proband's family, a previously reported mutation, *c.2970\_2972delAAAT* was identified. Similarly to our case, it was reported in connection with atypical NF with CALS with no other signs of clinical disease. In the second family,

a new mutation, *c.3103delAinsTTTC*, segregating with CALS was identified. **Conclusion:** Two different *NF1* mutations were found in two families with atypical NF manifested by multiple CALS not accompanied by other appa-

rent signs of clinical disease. One of them was identified as a new mutation. The result show that patients with multiple CALS with apparently no other signs of NF should be examined for *NF1* mutations.

#### P11.110

#### NF1 microduplication in two identical twins: description of the phenotype and comparison with previous described cases

O. García Campos<sup>1</sup>, P. García Murillo<sup>2</sup>, A. Verdú Pérez<sup>1</sup>, C. Catalí<sup>2</sup>;

<sup>1</sup>Neuropediatric service, Hospital Virgen de la Salud, Toledo, Spain, <sup>2</sup>Genetic department, Hospital Virgen de la Salud, Toledo, Spain.

**Introduction:** NF1 deletions are a well-known cause of Neurofibromatosis type 1, due mostly to non-allelic homologous recombination between low-copy repeats on the flanking regions. Despite this mechanism, very few cases of NF1 duplication have been described.

**Materials and Methods:** Two identical twins came to the attention of the neuropediatric service for developmental and speech delay and minor dysmorphic traits. Additional clinical and instrumental studies were performed. Genetic round-up included standard karyotype and CGH-array. Review of the literature was performed and we attempted comparison of the phenotype of the present cases with similar ones.

**Results:** Array-CGH detected a small 17q11.2 duplication involving the entire NF1 gene in one twin. MLPA study confirmed the presence of the duplication in her sister. Systematic review of published cases allowed oriented exploration in search of previously described features. We detected partial discordance of the phenotype, i.e. normal enamel and presence of some café-au-lait spots which has not been described before.

**Conclusions:** Phenotypes associated to NF1 duplication are highly variable and only few unspecific features like global developmental delay are shared by patients. Differences are probably due to small differences in additional microduplicated genes, while other features could be age-related. Extensive follow-up is warranted and, although clinically distinct from its reciprocal NF1 syndrome, careful evaluation of neurocutaneous symptoms is suggested.

#### P11.111

#### Molecular screening of NFIX in patients with Sotos-like overgrowth (Malan syndrome): 4 new mutations affecting the DNA-binding domain

I. Dapía, J. Tenorio, G. Gordo, P. Arias, T. Consortium, P. Lapunzina;

Medical and Molecular Genetics Institute (INGEMM), Madrid, Spain.

**Introduction:** The Nuclear-Factor I-X (NFIX) is a member of the nuclear factors-I (NFI) family proteins, involved in gene expression regulation. *De novo* heterozygous variants in NFIX may cause two different syndromes: Marshall-Smith syndrome (MSS; MIM #602535) or a less severe Sotos-like phenotype, recently proposed to be referred as Malan syndrome (MIM #614753). The overgrowth phenotype (Malan syndrome) has recently been associated to NFIX haploinsufficiency due to point mutations clustered around exon 2 (which encodes the DNA-binding domain) or due to whole gene deletions. Frameshift and splice-site variants around exons 6-8 are thought to be causing a more severe phenotype as they may escape nonsense-mediated RNA decay. To date 25 mutations have been described in association to the MSS phenotype. Only 20 have been reported in relation to the overgrowth phenotype so far.

**Methods:** We performed molecular analysis of the NFIX gene by conventional sequencing in a group of patients with overgrowth features but unclear clinical diagnosis. The effect of the mutations was predicted "in silico" by different bioinformatics tools.

**Results:** 5 heterozygous mutations in the NFIX DNA-binding domain were identified, 4 of them not previously reported. The patients showed some Sotos-like clinical features but no NSD1 abnormalities. All patients presented a significant delay in speech.

**Conclusions:** Genetic testing of NFIX should be considered in patients with Sotos-like features but no NSD1 alterations. To date the number of patients suffering from NFIX-related diseases is scant, thus further studies will be necessary in order to establish a specific clinical phenotype.

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#### P11.112

#### Clinical exome sequencing: Improving genetic diagnosis of complex diseases

A. Arteche<sup>1</sup>, L. Rausell<sup>1</sup>, M. Galvez<sup>2</sup>, M. Bermejo<sup>1</sup>, M. Lazaro<sup>1</sup>, M. Molero<sup>1</sup>, A. Gomez<sup>1</sup>, L. Cabrera<sup>1</sup>, P. Marín-García<sup>1</sup>, C. Ruiz<sup>1</sup>, J. Garcia-Planells<sup>1</sup>, M. Garcia-Hoyos<sup>1</sup>;

<sup>1</sup>Instituto de Medicina Genómica (IMEGEN), Paterna, Spain, <sup>2</sup>Gencellpharma, Bogota, Colombia.

The human exome represents 1-2% of the entire human genome and is estimated to contain 85% of the mutations causing Mendelian diseases. Next generation sequencing (NGS) offers rapid sequencing of the whole-exome compared to traditional molecular testing. This undoubtedly, contributes to significant advances in the study of etiology, origins, and causes of complex genetic diseases. NGS gives us the possibility not only to make previously unthinkable genetic diagnosis but also to reduce costs without affecting accuracy.

In this study, we present the genetic characterization of a patient with heterogeneous clinic using whole-exome sequencing (WES).

Our patient is a 12 years-old girl (46, XX), phenotypically characterized by precocious aging since her childhood, hyperelasticity, cutis laxa, and increased susceptibility to fractures. WES was carried out using the TruSight One kit (Illumina) that targets the coding regions of >4,800 genes associated with known clinical phenotypes (12Mb of the genome).

All variants, detected at >20X depth and with >30% of heterozygosity, were annotated and classified according ACMG guidelines, and were prioritized considering our patient's phenotype.

The exome analysis revealed a non-Previously reported nonsense change in homozygosity (c.190C>T; p.Gln64\*) in the gene GORAB (MIM#607983). This GORAB gene had been previously associated with Geroderma Osteodysplasticum (MIM#231070), an autosomal recessive disease that perfectly matches the phenotype of our patient.

The targeted exome sequencing that analyses all known clinically relevant genes, is a useful tool for the genetic diagnosis in patients with heterogeneous phenotype in whom no clear genetic causes were suspected.

#### P11.113

#### Genetic variants near the GREM1 locus are associated with a clinical subtype of nonsyndromic cleft lip and palate

A. C. Böhmer<sup>1,2</sup>, S. T. Ahmed<sup>3</sup>, N. B. Sangani<sup>2</sup>, S. Varghese<sup>3</sup>, J. Klamt<sup>1,2</sup>, H. Schuenke<sup>1,2</sup>, P. Gültepe<sup>1,2</sup>, A. Hofmann<sup>1,2</sup>, M. Rubin<sup>4</sup>, K. A. Aldhorae<sup>5</sup>, R. P. Steegers-Theunissen<sup>6</sup>, A. Rojas-Martinez<sup>7</sup>, R. Reiter<sup>8</sup>, G. Borck<sup>9</sup>, M. Knapp<sup>10</sup>, M. Nakatomi<sup>11</sup>, D. Graj<sup>12,13</sup>, E. Mangold<sup>1</sup>, H. Peters<sup>3</sup>, K. U. Ludwig<sup>1,2</sup>;

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life&Brain Center, University of Bonn, Bonn, Germany, <sup>3</sup>Institute of Genetic Medicine, Newcastle University, International Centre for Life, Newcastle upon Tyne, United Kingdom, <sup>4</sup>Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, Ferrara, Italy, <sup>5</sup>Orthodontic Department, College of Dentistry, Thamar University, Thamar, Yemen, <sup>6</sup>Department of Epidemiology, Radboud University Medical Center, Nijmegen, Netherlands, <sup>7</sup>Department of Biochemistry and Molecular Medicine, School of Medicine, and Centro de Investigación y Desarrollo en Ciencias de la Salud, Universidad Autónoma de Nuevo Leon, Monterrey, Mexico, <sup>8</sup>Department of Otolaryngology - Head and Neck Surgery, Section of Phoniatrics and Pedaudiology, University of Ulm, Ulm, Germany, <sup>9</sup>Institute of Human Genetics, University of Ulm, Ulm, Germany, <sup>10</sup>Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany, <sup>11</sup>Division of Anatomy, Kyushu Dental University, Kitakyushu, Japan, <sup>12</sup>Orofacial Development and Regeneration, Institute of Oral Biology, Center for Dental Medicine, University of Zürich, Zürich, Switzerland, <sup>13</sup>Departments of Dentistry and Medical Genetics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.

Nonsyndromic clefts are a common birth defect of multifactorial etiology. The most common type is cleft lip, which occurs with or without cleft palate (nsCLP and nsCLO, respectively). The genetic architecture of both types is not yet fully understood.

Here we performed a meta-analysis on genetic and clinical data from three large cohorts and identified strong association between a region on chromosome 15q13 and nsCLP ( $P=8.13 \times 10^{-14}$  for rs1258763; relative risk (RR): 1.46, 95% confidence interval (CI): 1.32-1.61)) but not nsCLO ( $P=0.27$ ). The 5 kb region of strongest association maps intergenically between Gremlin-1 (GREM1) and Formin-1 (FMN1) genes. GREM1 is an antagonist in the BMP4 pathway which has been implicated in facial genesis. Based on analyses of the murine Grem1 expression pattern during embryonic craniofacial development, we further subdivided the nsCLP patient cohort and observed a more than two-fold increase in risk for patients displaying clefts of both the lip and soft palate but with an intact hard palate (RR: 3.76, 95% CI: 1.47-9.61,  $P_{diff}<0.05$ ). Notably, in four of six multiplex-families with rare mutations in GREM1, the rare allele also co-segregated with the clinical subtype. *In silico* annotation such as chromatin interaction analyses further support GREM1 as plausible candidate gene at 15q13.

This study identified a non-coding region at 15q13 as the second, genome-

wide significant locus for nsCLP, after 13q31. Moreover, the data suggest a genetic contribution to a rare clinical nsCLP entity which specifically involves clefts of the lip and the soft palate which develop at different embryological time points.

### P11.114

#### Novel variants in "old" and "new" genes in patients with primary clinical diagnosis of Noonan syndrome.

**M. Gos<sup>1</sup>, A. Landowska<sup>1</sup>, A. Abramowicz<sup>2</sup>, S. Rzońca<sup>1</sup>, J. Sota<sup>1</sup>, T. Gambin<sup>2</sup>, S. Fahiminiya<sup>3</sup>, J. Kłapecki<sup>1</sup>, N. Beznakow<sup>1</sup>, A. Kukowska-Kaźmierczak<sup>1</sup>, J. Pilch<sup>2</sup>, M. Bodzioł<sup>3</sup>, J. Wierzbka<sup>4</sup>, A. Jakubik-Tomaszuk<sup>2</sup>, R. Śmigiel<sup>8</sup>, R. Posmyk<sup>5</sup>, M. Piotrowicz<sup>10</sup>, E. Obersztyn<sup>1</sup>, J. Majewski<sup>3</sup>, J. Bal<sup>1</sup>;**

<sup>1</sup>Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland,

<sup>2</sup>Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland,

<sup>3</sup>Department of Human Genetics, McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada, <sup>4</sup>Department of Pediatric Neurology, Medical University of Silesia, Katowice, Poland, <sup>5</sup>Department of Neurology, Jagiellonian University School of Medicine, Kraków, Poland, <sup>6</sup>Department and Clinic of Pediatrics, Hematooncology, Oncology and Endocrinology, Department of General Nursing, Medical University of Gdańsk, Gdańsk, Poland, <sup>7</sup>Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok, Białystok, Poland, <sup>8</sup>Department of Social Pediatrics, Wrocław Medical University, Wrocław, Poland, <sup>9</sup>NZOZ Genetics, Center for Clinical Genetics, Białystok, Poland, <sup>10</sup>Department of Genetics, Polish Mother's Memorial Hospital - Research Institute, Łódź, Poland.

Noonan syndrome (NS) is a multisystem disorder caused by mutations in genes encoding elements of RAS/MAPK pathway. The aim of our study was the identification of the molecular defect underlying NS phenotype in Polish patients. The classic Sanger sequencing and next generation sequencing techniques were applied to analyze only selected genes or exome /clinical exome, respectively.

The mutations in *PTPN11*, *SOS1*, *RAF1* and *RIT1* genes were identified in 115, 23, 16 and 7 patients, respectively. Among the identified mutations, several unpublished before were found including: familial variant in *SOS1* - p.Thr734Pro and *de novo* variants in *RAF1*: p.Gly169Arg and p.Gly361Ala. Also two new variants in *RIT1* were found: p.Gly31Arg and p.Lys23Glu in residues affecting protein structure or interaction with GTP/GDP, respectively. Further Sanger sequencing of selected genes revealed the presence of mutation in *KRAS* and *NRAS* genes in 3 and 2 patients, respectively. A novel *de novo* variant p.Glu153Lys in *NRAS* gene was identified.

Till now, the exome/clinical exome sequencing performed for 54 patients allowed to identify molecular cause of the disease in 20 (37%) patients, including variants in genes related to RAS/MAPK pathway (eg. p.Arg366Ter variant in *RASA2*), to other dysmorphic clinical entities like Kabuki syndrome (eg. *de novo* c.4032\_4034+1del variant in *KDM6A*) or novel NS candidate genes (eg. familial variant p.Ser247Asn in *LZTR1*). The application of sequencing techniques allows to identify novel potentially pathogenic variants that should be tested with functional (including *in silico*) studies and/or co-segregation analysis.

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### P11.115

#### Resolving molecular basis of novel phenotype: recessive, dominant and *de novo* disorders segregating in a consanguineous family

**P. Le Quesne Stabej<sup>1</sup>, H. Williams<sup>1</sup>, C. James<sup>1</sup>, L. Ocaka<sup>1</sup>, M. Tekman<sup>2</sup>, H. C. Stanescu<sup>2</sup>, R. Kleta<sup>2</sup>, Division of Medicine, UCL, D. Morrogh<sup>3</sup>, A. Calder<sup>4</sup>, M. Bitner-Glindzic<sup>2</sup>;**

<sup>1</sup>Genetics and Genomic Medicine, UCL Institute of Child Health, London, United Kingdom,

<sup>2</sup>Division of Medicine, UCL, London, United Kingdom, <sup>3</sup>NE Thames Regional Genetics Laboratory Service, London, United Kingdom, <sup>4</sup>Radiology Department, Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom.

We report a consanguineous family with four offspring. The mother has non-syndromic hearing loss (NSHL), 1<sup>st</sup> child has NSHL and craniometadial-physeal dysplasia (CMDD) - wormian bone type, 2<sup>nd</sup> child has NSHL only, 3<sup>rd</sup> child suffers from significant developmental delay with microcephaly in addition to NSHL and 4<sup>th</sup> child has CMDD - wormian bone type only. We performed whole exome sequencing (WES), linkage analysis and array Comparative Genomic Hybridisation (CGH) on all family members. There is a strong history of hearing loss on the mother's side of family. She is married to her 1<sup>st</sup> cousin with normal hearing. Whole exome sequencing (WES) identified a deafness-causing splice variant in an autosomal recessive deafness gene *PZD7* which resides in a linkage region (LOD score 3.6). In the two children diagnosed with a very rare skeletal phenotype (CMDD - wormian bone type), WES unexpectedly disclosed a heterozygous *COL1A1* variant shared between the two affected sibs as the cause of skeletal phenotype. The variant has been previously described to cause two skeletal phenotypes different to the phenotype in this family and was inherited from the apparently

unaffected mosaic father in an autosomal dominant fashion. This is the first report of molecular diagnosis made for CMDD - wormian bone type. Array-CGH detected a *de novo* unbalanced translocation as the cause of microcephaly with severe developmental delay in the 3<sup>rd</sup> child. WES analyses, linkage and array-CGH enabled accurate molecular diagnosis of three distinctive phenotypes segregating as a dominant, recessive or occurring *de novo* in one consanguineous family.

### P11.116

#### Oculo-auriculo-vertebral spectrum: High-Throughput Analysis

**E. Rubinato<sup>1</sup>, D. Vozzi<sup>1</sup>, I. Gandin<sup>1</sup>, A. Morgan<sup>1</sup>, A. D'Eustacchio<sup>2</sup>, M. La Bianca<sup>2</sup>, V. Pecile<sup>2</sup>, P. Gasparini<sup>1</sup>, F. Faletra<sup>2</sup>;**

<sup>1</sup>Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy, <sup>2</sup>Institute for Maternal and Child Health IRCCS, Burlo Garofolo, Trieste, Italy.

Oculo-auriculo-vertebral spectrum (OAVS) is a developmental disorder affecting structures derived from the first and second branchial arches during blastogenesis. The phenotype is clinically heterogeneous and it is typically characterised by craniofacial anomalies (ear malformations, hemifacial microsomia, mandible anomalies, orofacial cleft), hearing loss, ocular defects (epibulbar dermoids, microphthalmia) and vertebral defects. There is also a high rate of associated anomalies in other organs/systems such as heart defects, renal and central nervous system malformations. Both genetic and environmental factors are thought to contribute to the OAV spectrum, however, the mechanism and etiology are still poorly understood. Linkage and array analysis have detected several candidate loci for this genetically heterogeneous condition (including deletions, duplications, unbalanced translocations, partial trisomies of chromosomes 7, 8, 9, 10p, 22), but no recurrent chromosomal abnormalities have been identified so far.

The aim of our study is to identify candidate genes potentially involved in the pathogenesis of this condition. To achieve our goal we selected five unrelated patients presenting with the classical OAVS phenotype and without pathogenic Copy Number Variations (CNVs) detected by High-Density SNP-array (HumanOmniExpress BeadChip, Illumina, CA). Then, Whole Exome Sequencing analysis (Ion Proton™-LifeTechnologies) was carried out in all patients. CNV analysis was carried out by CoNIFER (<http://conifer.sourceforge.net>) with a confidence value  $\geq 1.5$ . Preliminary data shows no possibly pathogenic variants in genes shared by at least three out of five patients. Further analysis are currently undergoing. Updated data will be presented and discussed.

### P11.117

#### Two novel *MID1* mutations and the variable expression of developmental delay in X-linked Opitz G/BBB syndrome

**N. Maia<sup>1,2</sup>, M. Nabais Sá<sup>3,2</sup>, N. Tkachenko<sup>2</sup>, G. Soares<sup>2</sup>, I. Marques<sup>1,2</sup>, A. M. Fortuna<sup>3,2</sup>, R. Santos<sup>1,2,4</sup>, A. P. M. de Brouwer<sup>5</sup>, P. Jorge<sup>1,2</sup>;**

<sup>1</sup>Unidade de Genética Molecular, Centro de Genética Médica Jacinto de Magalhães (CGMMJ), Centro Hospitalar do Porto, Porto, Portugal, <sup>2</sup>Unidade Multidisciplinar de Investigação Biomédica (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal, <sup>3</sup>Unidade de Genética Médica, Centro de Genética Médica Jacinto de Magalhães (CGMMJ), Centro Hospitalar do Porto, Porto, Portugal,

<sup>4</sup>UCIBIO/REQUIMTE, Departamento de Ciências Biológicas, Faculdade de Farmácia,

Universidade do Porto, Porto, Portugal, <sup>5</sup>Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, Netherlands.

X-linked Opitz G/BBB syndrome (XLOS) is a midline multiple congenital condition characterized by facial dysmorphisms such as telecanthus/hypertelorism, broad nasal bridge and cleft lip/palate. Other features are variably present, including heart and anal defects, hypospadias, brain malformations and intellectual disability and/or developmental delay (ID/DD). Extensive inter and intra-familial phenotypic variability was previously described particularly regarding the presence of ID/DD in XLOS patients, even when caused by the same *MID1* mutation. *MID1* has E3 ubiquitin ligase activity that specifically targets protein phosphatase 2A for degradation. Controlling protein phosphatase 2A is essential for proper hippocampus development. To date over 90 different *MID1* mutations have been described and associated with the presence of ID/DD in XLOS patients.

We analyzed the coding sequence of the *MID1* gene in eight male patients, pertaining to six unrelated families, with a clinical suspicion of XLOS. Two novel variants, c.1656del and a *de novo* c.1215\_1228dup, were identified in four patients from two families. Interestingly, the patient with the *de novo* mutation did not present ID/DD, showing a global developmental quotient of 110.

A descriptive statistical analysis of the specific clinical features of our two index patients and of those described in the literature with genotypic data (n=103), revealed no correlation between ID/DD penetrance and type of

mutation or affected MID1 protein domain (missense mutations, in the latter). This could indicate absence of an effective phenotype-genotype correlation concerning ID/DD or alternatively insufficient data due to poorly described cognitive deficits in these patients (i.e. full IQ assessment is often missing).

#### P11.118

#### OFD1 in males: Congenital heart defects are included its phenotypic spectrum

*A. Bouman<sup>1</sup>, M. Alders<sup>1</sup>, L. van Leeuwen<sup>2</sup>, N. Thuijs<sup>3</sup>, M. van Maerle<sup>1</sup>;*

<sup>1</sup>AMC, Department of Clinical Genetics, Amsterdam, Netherlands, <sup>2</sup>AMC, Department of Obstetrics and Gynaecology, Amsterdam, Netherlands, <sup>3</sup>AMC, Department of Pathology, Amsterdam, Netherlands.

Oral-facial-digital syndrome type 1 (OFD1) is an X-linked dominant disorder caused by mutations in the *OFD1* gene. This condition is characterized by facial anomalies and abnormalities of oral tissues, digits, brain and kidneys. Almost all affected patients are female, as OFD1 is lethal in males mostly in the first or second trimester of pregnancy.

Although OFD1 is a disorder of the primary cilia, congenital heart defects are not frequently reported in both males and females with OFD1. Only a few cases were published this far, describing male OFD1 patients with a congenital heart defect.

Here, we present a case of an affected male fetus with a pathogenic hemizygous *de novo* mutation in OFD1 (c.2101C>T; p.(Gln701\*)). Ultrasound examination demonstrated severe hydrocephalus, hypoplastic cerebellum and hypoplastic left ventricle of the heart. The pregnancy was terminated at 16 weeks of gestation. Post-mortem examination of the fetus confirmed previous findings and also demonstrated polydactyly of both hands and one foot.

By presenting this case of a male fetus with confirmed OFD1 and a congenital heart defect, we would like to create awareness that congenital heart defects are included in the phenotypic spectrum of OFD1. This is especially of importance when prenatally multiple malformations are seen and the suspicion of OFD1 is raised, also when the family is unsuspected for OFD1. A clinical summary of this case and previous male OFD1 cases with a congenital heart defect will be demonstrated.

#### P11.119

#### LRP5-linked Osteoporosis-Pseudoglioma syndrome mimicking isolated microphthalmia

*S. Guntekin Ergun<sup>1</sup>, M. A. Ergun<sup>1</sup>, G. Gümüş Akay<sup>2</sup>, N. A. Akarsu<sup>3</sup>, F. E. Percin<sup>4</sup>;*

<sup>1</sup>Gazi University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey,

<sup>2</sup>Ankara University, Brain Research Centre, Ankara, Turkey, <sup>3</sup>Hacettepe University

Faculty of Medicine, Department of Medical Genetics, Gene Mapping Laboratory, Ankara, Turkey, <sup>4</sup>Gazi University Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey.

**Introduction:** Osteoporosis-Pseudoglioma syndrome (OPGG; MIM 259770) is a rare autosomal recessive disorder manifesting juvenile-onset osteoporosis and blindness. Affected individuals often have multiple bone fractures, particularly in spinal bones. Vision problems affect the retina and named as pseudoglioma since the conditions resemble an eye tumor known as retinal glioma. Mild intellectual disability, hypotonia, abnormal flexible joints or seizures are frequently associated with the disorder. It is caused by biallelic mutations in the LRP5. Here we reported a four generation consanguineous family in which three members, ranging between 19 and 28 years, were affected by bilateral isolated microphthalmia.

**Materials and Methods:** Homozygosity mapping and exome sequencing have been performed to clarify the genetic etiology of the bilateral isolated microphthalmia in this family.

**Results:** The exome sequencing revealed a homozygous splice-site mutation in LRP5 (c.2827+1G>A) in the index patient.

**Conclusions:** Although no osteoporosis or bone fracture history in any of the affected members, dual-energy X-ray absorptiometry revealed osteoporosis in the spine in two examined affected members. Two sisters with compound heterozygous mutations in LRP5: c.889dupA and c.2827+1G>A associated with bilateral retinal detachment, microphthalmic eye, retro-lental masses and reduced bone density have recently been reported. Thus, microphthalmia and reduced bone density observed in our family considered to be a component of OPGG syndrome. Taken together our study showed that isolated microphthalmia can be the prominent symptom in a subset of OPGG families and, even no associated findings observed, bone mineral density measurement is essential for appropriate genetic counselling in such cases.

#### P11.120

#### Analysis of overgrowth disorders through custom NGS panel of 183 genes (OGLYVAS)

*J. Tenorio, G. Gordo, I. Dapía, P. Arias, E. Vallespín, K. Ibañez, J. Silla, Á. Del Pozo, T. Consortium, P. Lapunzina;*  
INGEMM, Madrid, Spain.

**Introduction:** Overgrowth syndromes (OGS) comprise a group of conditions in which growth parameters are above of the mean and are usually accompanied by other clinical manifestations. In some cases, there is high overlapping between different OGS, making essential the molecular diagnosis.

**Material and Methods:** We have designed a custom panel of NGS in which we included: i) genes responsible for any OGS, ii) genes related to the pathways that leads to any disorder that resemble an OGS and iii) genes within a loci implicated in any overgrowth disorders described in the literature. Thus, 183 genes have been included. Eighteen patients, divided into those with clear phenotype but unconfirmed diagnosis (n=3) and those with non-syndromic overgrowth and intellectual disability (n=14) were tested. One sample with known mutation and phenotype has been included as an internal control.

**Results:** After variants filtering, fourteen mutations have been found in eleven unrelated patients, five of which are causative genes for well-known overgrowth syndromes. Otherwise, nine variants have been found in seven genes which are within a deletion or duplication syndrome with overgrowth and/or genes that act in several growth pathways but without a clear relationship between their mutations and phenotype abnormalities.

**Conclusions:** Custom NGS panel of genes of overgrowth disorders is an extremely cost-effective tool to diagnose those cases in which there is high clinical overlapping between different syndromes and also gives the possibility to find mutation in genes from growth pathways that could explain the phenotype in undiagnosed cases of non-syndromic overgrowth.

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#### P11.121

#### OVOL2 variants in a syndrome with cleft lip, colonic atresia and other congenital abnormalities

*P. J. G. Zwijnenburg, J. M. Weiss, H. J. Meijers-Heijboer, Q. Waisfisz;*  
VU university medical center, Amsterdam, Netherlands.

Ultrasound screening at 20+2 weeks showed a bilateral cleft lip in the fetus of a non-consanguineous couple. At 30+5 weeks, a boy was born with normal weight, height and OFC. He suffered from respiratory insufficiency, bilateral cleft lip-palate, a Peters anomaly, right sided aortic arch, severe tracheomalacia and atresia of the transverse colon. Karyotyping, SNP array and analysis of B3GALT1, CHD7 and ASXL1 was normal. He remained respiratory insufficient and died after 6 weeks.

Shortly after whole exome sequencing (WES) was initiated, mother was pregnant. At 17+1 weeks an ultrasound showed a bilateral cleft lip without other abnormalities and the pregnancy was terminated. A female fetus was born with a bilateral cleft lip-palate. Autopsy showed malrotation and a short ascending colon, cysts in the intestinal wall, uterus bicornis and hemivertebra.

WES analysis revealed compound heterozygous variants in OVOL2, c.447C>A p.(Cys149\*) and c.538del p.(Cys180Alafs\*15) in both sibs. p.(Cys149\*) is a nonsense variant in the second last exon, most likely resulting in nonsense mediated mRNA decay. p.(Cys180Alafs\*15) causes a premature stopcodon in the last exon and therefore predicted not to result in nonsense mediated decay. To our knowledge, this is the first report on nonsense variants in OVOL2 in humans. OVOL2 is considered to play an important role in early embryonic development; Ovol2-deficient mice display severe defects including underdeveloped branchial arches, abnormal gut morphology and aberrant vascularization.

Therefore, we consider the nonsense variants in OVOL2 likely to be causative for this syndrome of bilateral cleft lip-palate, colonic atresia and other congenital malformations.

#### P11.122

#### Diagnosis of Pallister Killian syndrome by aCGH analysis

*Z. Yilmaz Celik<sup>1</sup>, Y. K. Terzi<sup>1</sup>, I. Erol<sup>2</sup>, F. I. Sahin<sup>1</sup>;*

<sup>1</sup>Baskent University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Baskent University Faculty of Medicine Department of Pediatric Neurology, Ankara, Turkey.

**Introduction:** Array comparative genomic hybridization (aCGH) is a high resolution cytogenetic tool for detection of segmental genomic copy number variations (CNVs). Large numbers of genetic syndromes associated with loss or gain of human genome have been diagnosed by this method. Here we report a one year old girl consulted due to dysmorphic face and hypotonia. Although, conventional cytogenetics revealed a normal karyotype, we iden-

tified a 12p11.1p13.33 copy number gain by aCGH.

Materials and methods: Peripheral blood lymphocytes were used for DNA isolation. aCGH analysis was carried on by Roche NimbleGen 630K array ISCA Plus Cytogenetics array and was analyzed by Nexus 7.5 software. The LSI TEL/AML1 ES Dual Color translocation probe was used for detection of tetrasomy 12p mosaicism.

Results: The girl was the second child of nonconsanguineous parents. She had dysmorphic features including frontal bossing, round face, sparse scalp hair, low-set abnormal ears, wide nasal bridge, short nose, long filtrum, thin upper lip, everted/thick lower lip, short neck. We detected copy number gain on short arm of the chromosome 12 encompassing about 34.5 Mb by aCGH. This data was also approved by FISH. However, FISH analysis revealed that the patient carried 10% mosaic i(12p) in lymphocyte cultures.

Conclusions: As a result the patient was diagnosed as Pallister-Killian syndrome. Peripheral blood lymphocytes were utilized for FISH to confirm diagnosis, so we did not perform invasive skin biopsy. This finding approved the previous reports suggesting that aCGH analysis may be enough to make diagnosis Pallister-Killian syndrome without any invasive applications.

#### P11.123

#### Postnatal diagnosis of Pallister-Killian Syndrome after non-invasive prenatal testing

D. Wahl<sup>1</sup>, W. Schenk<sup>2</sup>, U. Heinrich<sup>3</sup>, I. Rost<sup>3</sup>;

<sup>1</sup>Practise, 86150 Augsburg, Germany, <sup>2</sup>Childrens' Hospital, 86152 Augsburg, Germany,

<sup>3</sup> MVZ, 82152 Martinsried, Germany.

We report on a newborn girl, the second child of healthy parents, born after an uneventful pregnancy. In order to avoid the risk of invasive prenatal diagnostics, a non-invasive prenatal test was performed, excluding trisomies 13, 18 and 21. Furthermore, high-resolution ultrasound at 20-22. week's gestation showed normal results. The parents felt save and were looking forward to give birth to a healthy child. Immediately after birth an increased muscle tone, as well as an anteriorly positioned anus, a duplication of the right great toe and dysmorphic facial features were evident. Audiometry showed moderate hearing disturbance, ultrasound of the heart revealed an atrial septal defect. The sorrowful parents were convinced by the genetic counselor, that further genetic investigations were indicated and agreed to Array-CGH analysis. A mosaic tetrasomia 12p - causative for Pallister-Killian Syndrome - was detected. All symptoms of the child could be assigned to this diagnosis. Further involvement of eyes, skin and lungs could be excluded. The diagnosis of this severe and rare chromosomal disorder was explained to the parents, and their question, whether there had been a diagnostic failure during pregnancy was appeased. Meanwhile the parents feel content with their 6 months old girl and accept her slow but continuous development as well as the decisions they had taken during pregnancy, having undergone various steps of trauma processing and supervision.

#### P11.124

#### Deletion of *PDKCC* gene in a patient with meoxaial and preaxial polydactyly, complex cardiac defect and facial features of Pallister Hall syndrome

R. D. Clark;

Loma Linda University Medical Center, Loma Linda, CA, United States.

Deletion of the *PDKCC* gene (MIM 614150) at 2p21 has no known phenotypic consequence in humans. However, the mouse homolog of *PDKCC*, Vlk, is a hedgehog target that interacts with Gli3 to regulate chondrocyte differentiation. A patient with suspected Pallister-Hall syndrome had a deletion at 2p21 that included only the *PDKCC* gene suggesting that haploinsufficiency for this gene may mimic a mutation in *GLI3*. The mother was a 33 year old obese G8P4034 with bicornuate uterus and diet controlled gestation diabetes mellitus A1 (HgA1c 5.5) from 30 weeks gestation. The patient was prenatally diagnosed with transitional AV canal, right ventricular hypoplasia, cleft mitral valve with regurgitation and pulmonary valve hypoplasia. Amniocentesis revealed a normal karyotype and microarray. Birth weight was 3476 grams, head circumference was 36.5 cm (98<sup>th</sup> percentile). After delivery other anomalies were: macrocephaly, central polydactyly of the left hand, preaxial polydactyly of the halluces, hypoplastic nails, facial dysmorphism, absent gag reflex, increased tone of the upper extremities. Pallister Hall syndrome was suspected clinically but the *GLI3* gene analysis was normal. MRI of the brain showed mildly enlarged ventricles and sulci with irregular gyri. The prenatal microarray report was amended after delivery, identifying a 94 kb deletion at 2p21 of the *PDKCC* gene. Parental microarrays are pending. This is the first report of an abnormal phenotype in a human associated with a deletion of *PDKCC* and it suggests that this gene functions in the same pathway as *GLI3*.

#### P11.126

#### Hallmark features of CLAPO syndrome in two patients with PROS phenotype and confirmed PIK3CA somatic mosaic mutations

V. Martinez-Glez<sup>1</sup>, G. Gordo<sup>1</sup>, L. Rodriguez-Laguna<sup>1</sup>, G. Herranz<sup>1</sup>, S. Garcia-Minaur<sup>1</sup>, F. Santos-Simarro<sup>1</sup>, J. Lopez-Gutierrez<sup>2</sup>, P. Lapunzina<sup>1</sup>;

<sup>1</sup>INGEMM-CIBERER-IdiPaz-Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>Vascular Anomalies Center, Plastic Surgery, Hospital Universitario La Paz, Madrid, Spain.

PROS (PIK3CA-Related Overgrowth Spectrum) comprises a group of segmental overgrowth syndromes caused by somatic mutations in the *PIK3CA* gene, including previously considered separate syndromes such as Megalencephaly-Capillary malformation (MCAP) and CLOVES (Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, And Skeletal/Spinal abnormalities). However, as somatic mosaicism makes clinical expression variable in severity and location, the complete phenotypic spectrum in PROS is still to be elucidated. On the other hand, CLAPO syndrome (Capillary malformation of the lower lip, Lymphatic malformation of the face and neck, Asymmetry and Partial/generalized Overgrowth), is another rare disease in this group of the overgrowth syndromes, with a highly similar phenotype to PROS, but of unknown cause, although somatic mosaicism is also suspected. Here we describe two patients with multiple clinical and radiological features associated to PROS and confirmed *PIK3CA* mosaic mutations. These patients also show two manifestations not described as part of the PROS spectrum and considered to be the hallmarks of CLAPO syndrome —Capillary malformation of the lower lip and Lymphatic malformation of the face and neck—. Therefore, we report a phenotypic overlap between these two entities and suggest a possible pathogenic association, which should be further explored in patients with CLAPO.

#### P11.127

#### In vitro studies with an AKT inhibitor, ARQ092, provide evidence for a new and more effective therapeutic option in PIK3CA Related Overgrowth Spectrum (PROS) patients

D. Loconte<sup>1</sup>, V. Grossi<sup>1</sup>, S. Di Tommaso<sup>1</sup>, C. Ranieri<sup>1</sup>, R. Bagnulo<sup>1</sup>, A. Pesarico<sup>1</sup>, G. Forte<sup>2</sup>, P. Sanese<sup>1</sup>, A. Giglio<sup>3</sup>, Y. Yu<sup>4</sup>, G. Abbadessa<sup>5</sup>, C. Simone<sup>1,2</sup>, N. Resta<sup>1</sup>;

<sup>1</sup>Division of Medical Genetics; Department of Biomedical Sciences and Human Oncology; University, Bari, Italy, <sup>2</sup>Cancer Genetics Laboratory, IRCCS "S. de Bellis", Castellana Grotte (BA), Italy, <sup>3</sup>Department of Emergency and Organ Transplantation-Urology, Andrology and Kidney Transplantation Unit, Bari, Italy, <sup>4</sup>Biology, ArQule, Inc., Burlington, MA, United States, <sup>5</sup>Clinical Development, ArQule, Inc, Burlington, MA, United States.

Introduction: In PROS, PIK3CA germline and postzygotic mutations constitutively activate the PI3K/AKT/mTOR pathway causing congenital segmental overgrowth, while are absent in surrounding healthy tissues. mTOR inhibitors are empirically tested in these patients. We aim to assess the effects on PROS patient-derived cells of pathway blockage upstream of mTOR by the potent and selective allosteric AKT inhibitor ARQ 092, an experimental drug with activity and long-term tolerability in many cancer patients, being also tested in Proteus patients.

Materials and methods: We performed targeted deep sequencing of pathway genes in six PROS patient-derived cells to identify causative mutations and immunoblots to assess the phosphorylation status of AKT and its downstream targets (pS6, pPRAS40, pFOXO3a, pBAD, pGSK3α-β). Anti-proliferative effect of ARQ 092 and PI3K/AKT/mTOR inhibitors (wortmannin, LY294002, syrolimus) was evaluated, with or without serum, in PROS cells from six patients.

Results: ARQ 092 potently inhibited Akt signaling and exerted a strong anti-proliferative effect by inducing cell death more efficiently than comparators, with 50% of surviving cells after 60 hours of treatment with 5 uM dose.

Conclusions: Our data show that PROS cells are 'addicted' to AKT and PROS treatment benefits more of AKT than mTOR inhibition. Clinical development of ARQ 092 in PROS patients is warranted.

#### P11.128

#### PROS spectrum: Two different germline and somatic mutations in PIK3CA in a patient with segmental overgrowth, hemimegalencephaly and vascular malformations

F. López Grondona<sup>1</sup>, P. Abenia Ausón<sup>2</sup>, P. Fernández Álvarez<sup>1</sup>, I. Valenzuela Palafoll<sup>1</sup>, M. del Campo Casanelles<sup>1</sup>, V. García Patos<sup>1</sup>, A. Cueto González<sup>1</sup>, T. Vendrell Bayona<sup>1</sup>, M. Borregan Prats<sup>1</sup>, L. Martínez Ribot<sup>1</sup>, E. Tizzano Ferrari<sup>1</sup>;

<sup>1</sup>Vall d'Hebron Hospital, Barcelona, Spain, <sup>2</sup>Hospital Universitario Miguel Servet, Zaragoza, Spain.

In 2015 the acronym PROS (PIK3CA - Related Overgrowth Spectrum) was defined to encompass syndromes characterized by overgrowth, megalencephaly, vascular and skin malformations related with somatic mutations in the gene phosphatidylinositol-3-kinase (PIK3CA). We present a patient with

a germline mutation present in blood, skin, and buccal mucosa and a second somatic mutation present only in overgrowth affected tissues.

A 4-year-old boy with congenital asymmetric macrocephaly, segmental overgrowth and vascular malformations of skin was initially diagnosed as Proteus syndrome (PS). Studies in PTEN and AKT1 were negative and was referred to our Center.

He showed right hemimegalencephaly, polymicrogyria and schizencephaly, right hemifacial overgrowth, vascular malformations, segmental overgrowth in lower extremities and upper limb hypotrophy. Suspicion of related PROS was made and PIK3CA mutations were investigated in blood samples, skin biopsy from two areas of hypertrophy and buccal mucosa.

Results: We amplified exons 8-10 and 21 of PIK3CA (NG\_012113.2, NM\_006218.2) as hot spot of mutations.

The patient was heterozygous for the germline mutation c.1634A>C, (p.Glu545Ala) + c.1658\_1659 delGTinsC (p. Ser553Thrfs\*7) in all the analyzed tissues. This complex allele was previously described as pathological and associated with Cowden and Cowden-like Syndromes. DNA obtained from the hypertrophic areas revealed a somatic mutation c.3139C>T (p.His 1047Tyr) previously described in Megalencephaly-capillary malformation (MCAP) patients.

PIK3CA mutations have been described as somatic in mostly PROS cases, except for a few patients with megalencephaly-capillary malformation and germline mutations. This is the first report of two PIK3CA mutations supporting the double hit hypothesis on the genesis of these phenotypes.

#### P11.129

#### Hypomyelinating leukodystrophy-8 and cerebellar hypoplasia with endosteal sclerosis are allelic disorders caused by mutations in POLR3B

*M. García-Murias<sup>1,2</sup>, M. Sobrido<sup>1,2,3</sup>, A. Schlüter<sup>2,4</sup>, A. Pujol<sup>2,4</sup>, C. Castro-Fernández<sup>1,5</sup>, F. Barros<sup>1,2,3</sup>, P. Blanco-Arias<sup>1,2,3</sup>, P. Cacheiro<sup>1,5</sup>, S. Beltrán<sup>6,7</sup>, M. Gut<sup>6,7</sup>, S. Laurie<sup>8,7</sup>, A. Carracedo<sup>2,3,9</sup>, J. Eirís-Puñal<sup>9</sup>, B. Quintáns<sup>1,2</sup>;*

<sup>1</sup>Instituto de Investigación Sanitaria, Santiago de Compostela, Spain, <sup>2</sup>CIBERER, Madrid, Spain, <sup>3</sup>Fundación Pública Gallega de Medicina Xenómica, Santiago de Compostela, Spain, <sup>4</sup>Laboratory of Neurometabolic Diseases, ICREA, IDIBELL, Barcelona, Spain, <sup>5</sup>USC, Santiago de Compostela, Spain, <sup>6</sup>CNAG-CRG, Centre for Genomic Regulation, Barcelona Institute for Science and Technology, Barcelona, Spain, <sup>7</sup>Universitat Pompeu Fabra, Barcelona, Spain, <sup>8</sup>Complejo Hospitalario Universitario, Santiago de Compostela, Spain.

**BACKGROUND:** cerebellar hypoplasia with endosteal sclerosis (OMIM 213002) is a disease of unknown cause with a few cases published in the scientific literature. Hypomyelinating leukodystrophy-8 (HLD8) with hypodontia and hypogonadotropic hypogonadism (also known as 4H syndrome) is caused by mutations in POLR3B. **AIM:** To describe two patients from the same family with cerebellar hypoplasia and mutations in POLR3B. **CASE REPORT:** A 22 years-old male was diagnosed at the age of 5 with cerebellar hypoplasia and endosteal sclerosis. He had microcephaly, short stature, myopia, dental dysplasia, hypogonadism, scoliosis, ataxia, intention tremor and psychomotor retardation. His 5-years old cousin had myopia, astigmatism, psychomotor retardation, microcephaly, hypodontia, and ataxia. Her brain MRI showed cerebellar hypoplasia and hypomyelination. Endosteal sclerosis was not present in the girl. Whole exome sequencing was carried out in both patients and the unaffected parents of one of them. The patients were compound heterozygous for two likely pathogenic variants in POLR3B: NM\_018082.5:c.[1568T>A]+[2974G>A]; NP\_060552.4:p.[V523E]+[G992S]. The p.V523E variant has been described in several patients, while the p.G992S variant was not reported previously. All the heterozygous carriers in the family were asymptomatic. **CONCLUSIONS:** Mutations in POLR3B cause cerebellar hypoplasia with endosteal sclerosis. We show that this disease is part of the phenotypic spectrum of the 4H syndrome. This study adds another example to consolidate the cost-effectiveness of whole exome sequencing for the diagnosis of rare diseases, especially when several affected individuals can be analyzed. **Funding:** 300 exomes to elucidate rare disease-CNAG; FIS PI12/00742.

#### P11.130

#### Progressive osseous heteroplasia (POH) and pseudohypoparathyroidism type Ia (PHP1A) in two unrelated families with two novel mutations in GNAS gene

*A. Skórka<sup>1,2</sup>, D. Gieruszczak-Biakę<sup>1,2</sup>, E. Ciara<sup>2</sup>, K. Chrzanowska<sup>2</sup>, I. Garin<sup>3</sup>, G. Perez de Nanclares<sup>3</sup>;*

<sup>1</sup>Department of Pediatrics Medical University of Warsaw, Warsaw, Poland, <sup>2</sup>Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland,

<sup>3</sup>Molecular (Epi) Genetics Lab, Research Unit, Hospital de Txagorritxu, Vitoria-Gasteiz, Spain.

Introduction: GNAS heterozygous inactivating mutations cause PPHP/POH

or PHP1A depending on the parental allelic origin of the mutation (paternal or maternal, respectively). POH is characterized by progressive ossifications of dermal, skeletal muscle and deep connective tissues during childhood, whilst PHP1A is characterized by resistance to PTH in association with features of Albright Hereditary Osteodystrophy (AHO).

**Patients and Methods:** First family, 28 years old woman presented with cutaneous calcifications from early childhood followed by deep tissue tumors. Her daughter was neonatally diagnosed with congenital hypothyroidism, but as she grew features of AHO and PTH resistance were noted. In the second family, a 30 years old female presented with cutaneous calcification from infancy which then progressed into deep tissues. Her 3.5 years old son was diagnosed with congenital hypothyroidism, PTH resistance, AHO phenotype and he presented epilepsy with calcification of the basal ganglia. GNAS gene was sequenced in both families.

**Results:** Molecular analysis of GNAS gene revealed a novel missense mutation (p.Gln19\*) in exon 1, in the girl and mother of the first family. In the second family, a novel heterozygous missense mutation was identified in exon 6 of GNAS gene, p.Arg160Pro in the boy and her mother, confirming correlation with the disease.

**Conclusions:** These results further expand the spectrum of GNAS mutations associated with POH/PHP1A and underline the importance of identifying such genetic alterations to supplement clinical evaluation and genetic counseling.

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#### P11.131

#### Cerebral Neoplasms in Proteus syndrome: case report

*M. R. Pizzino<sup>1</sup>, I. Loddo<sup>2</sup>, M. Crapanzano<sup>1</sup>, C. Pidone<sup>1</sup>, M. C. Cutrupi<sup>1</sup>, S. Briuglia<sup>1</sup>, <sup>1</sup>Dipartimento di Paediatric Science Messina, Messina, Italy, <sup>2</sup>University of Messina, Messina, Italy.*

**Introduction:** Proteus syndrome (PS) is extremely rare disease with malformations and overgrowth of different tissues. Prognosis is severe with premature death. It is caused by mosaic mutation of (PI3K)-AKT signaling pathway. Features are asymmetrical overgrowth of limbs, hyperostosis, cerebriforme connective nevi, skeletal deformities, benign and malignant tumors, capillary vascular malformations and deep venous thrombosis.

**Case report:** We report a 15 years girl with a severe expression of PS. She had serious deformities of skull, thorax, arms, hands, feet, macrodactyly, scoliosis, various deep vein thrombosis, ovaries cystadenoma and bullous pulmonary disease. Then she showed progressive respiratory failure, behavior changes, depression, headaches, fatigues. Brain MNR showed severe hyperostosis and two lesions like meningioma, with significant compression on the midbrain, brain parenchyma and cerebral edema, hydrocephalus. The severe progression of lesions determined respiratory failure and patient death.

**Conclusion:** CNS malformations and meningioma have been previously reported in patients with PS. Because of brain tumor late diagnosis and severe complications, therapy has been extremely difficult and patient died. The PS prognosis varies depending on the severity of complications. Patients should be examined regularly and annual physical examination and radiography is also recommended. An appropriate follow up are most important, but extreme rarity of PS makes very difficult the multidisciplinary approach.

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#### P11.132

#### Exome sequencing uncovers homozygous mutation in PCDHB10 in two brothers with syndromic intellectual disability and palmoplantar lipomatosis

*V. López-González<sup>1,2</sup>, M. J. Ballesta-Martínez<sup>1,2</sup>, M. J. Sánchez-Soler<sup>1,2</sup>, L. Rodríguez-Peña<sup>1,2</sup>, B. Rodríguez-Santiago<sup>3</sup>, L. Armengol-Dulcet<sup>3</sup>, E. Guillén-Navarro<sup>4</sup>,*

<sup>1</sup>Sección de Genética Médica. Servicio de Pediatría. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, <sup>2</sup>Grupo Clínico Vinculado al Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER). Instituto de Salud Carlos III (ISCIII), Madrid, Spain, <sup>3</sup>qGenomics Laboratory, Barcelona, Spain, <sup>4</sup>Consejería de Sanidad, Murcia, Spain.

**Introduction:** Autosomal recessive inheritance accounts for 25% of intellectual disability (ID). Diagnosis can be challenging as clinical presentation is frequently undifferentiated. Whole exome sequencing (WES) is a powerful tool for gene discovery.

Clinical report: We report on two male siblings affected by syndromic ID, born to consanguineous parents of Roma origin, with a previous healthy daughter.

The patients are 15 and 5 years old. The eldest displays moderate ID, no expressive language, hand flapping, hearing loss, ventricular septal defect, vesicoureteral reflux, and a limp due to Perthes disease. The youngest presents with psychomotor delay, epilepsy and growth retardation. Their dysmorphic features include broad nasal bridge, wide mouth with a bow-shaped upper lip, large ears, sacral dimples and deep creases on palms and soles.

Results: WES revealed a homozygous stop variant c.T741A;p.Y247X in the PCDHB10 gene, validated by Sanger. This novel variant was not observed in the variant databases and segregated as expected within the family.

Discussion: We have identified a possible new syndrome characterized by ID, dysmorphic features and palmoplantar lipomatosis, caused by a novel stop homozygous mutation in a gene not previously reported as causal. Although further analysis is necessary, almost all protocadherin family genes are widely expressed in the brain and play important roles in its development and function, what could support its pathogenicity. Due to the association of ID and deep creases, Pierpont, Coffin-Siris and Wiedemann-Steiner syndromes should be regarded as differential diagnoses. Our report emphasizes the clinical utility of WES in patients with undiagnosed ID.

#### P11.133

#### Mutation analysis in the ABCC6 gene and genotype-phenotype correlations in the french cohort affected by pseudoxanthoma elasticum

**A. Legrand**<sup>1,2</sup>, **L. Cornez**<sup>3</sup>, **W. Samkari**<sup>1</sup>, **A. Venisse**<sup>4</sup>, **D. Germain**<sup>4</sup>, **J. Albuisson**<sup>1,2,5</sup>, **X. Jeunemaitre**<sup>1,2,5</sup>;

<sup>1</sup>Département de génétique, Hôpital Européen Georges Pompidou, APHP, Paris, France,

<sup>2</sup>Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France,

<sup>3</sup>Commissariat à l'énergie atomique et aux énergies alternatives, CEA Saclay, Gif-sur-Yvette, France, <sup>4</sup>Université de Versailles - St Quentin en Yvelines (UVSQ), Division of Medical Genetics, Montigny, France, <sup>5</sup>INSERM, U970, Paris centre de Recherche Cardiovasculaire-PARCC, Paris, France.

Introduction: Pseudoxanthoma elasticum (PXE) is a rare, autosomal recessive disorder, characterized by ectopic mineralization and fragmentation of elastic fibers of connective tissues, leading to skin, eye and cardiovascular system symptoms. PXE is caused by loss of function mutations in ABCC6, coding a membrane transporter of unknown function.

Methods: A comprehensive molecular analysis of 306 French PXE probands diagnosed using the Phenodex score, was performed (including Sanger sequencing of all exons, Long-range PCR focusing the recurrent multie exon deletion, and MLPA). Topography of the mutations was analysed, and genotype-phenotype correlation analysis was performed in patients with 2 mutations: group H (haploinsufficient, 2 predicted loss-of-function variants), group M (predicted residual conservation of the transporter function, 2 missense mutations) and group HM (one of each type of variants).

Results: Molecular analysis led to the identification of 142 distinct mutations with 66 novel mutations and a detection rate of 88%. Significant results were found: (1) Mutations distribution was specific to some regions and residues of the transporter ( $p=0,005$ ), (2) a more severe ocular and vascular phenotype exists in patients in groups H and HM than in group M ( $p=0,05$ ), (3) renal lithiasis and strokes, which are not included in the Phenodex score, were found respectively in 11 and 10% of cases.

Conclusion: This is the largest cohort ever published and characterized, and we propose a revised Phenodex classification, as well as personalized care and prevention based on the patients' molecular status, enhancing vascular follow-up in patients with complete haplo-insufficiency of the gene.

#### P11.134

#### Noonan Syndrome and RAS-MAPK pathway: a road map over the last 45 years in a tertiary hospital

**A. M. Cueto-González**, **T. Vendrell-Bayona**, **S. Boronat**, **M. Clemente**, **F. López-Grondona**, **M. Borregan-Prats**, **L. Martínez-Ribot**, **E. Tizzano-Ferrari**, **Hospital Vall d'Hebron, Barcelona, Spain**.

Germline mutations in genes of the RAS/MAPK pathway induced the activation of a cascade of events causing proliferation, differentiation, survival and cell death. Clinical consequences in patients including: short stature, congenital heart defects, facial dysmorphism, developmental delay and ectodermal anomalies among the most significant that are known as RASopathies. We compiled all patients diagnosed with RASopathies during the last 45 years in our Clinical Genetics Department to characterize their phenotype-genotype correlation.

In total we have diagnosed 144 patients with clinical features compatible with RASopathies. In 55 of them we found the causative molecular defect. In 37 patients mutations in PTPN11 were found (4 familial, and a Leopard phe-

notype in one case); 9 patients showed SOS1 mutations (2 familial); 3 had mutations in BRAF (all sporadic cases with phenotype cardio-facio-cutaneous); 1 in the NRAS; 2 in RAF1 (both sporadic); 1 in RIT1 (sporadic) and 1 in SHOC2 (sporadic). CGH array revealed one partial duplication in RAF1 (0,07Mb). A comprehensive phenotype-genotype correlation was performed. Noonan Syndrome is the most frequent diagnosed syndrome with characteristic features that make clinical diagnosis very accurate. However, many of that features overlap with clinical findings described in the other genes in the RAS/MAPK pathway making specific diagnosis more difficult.

#### P11.135

#### Spectrum of pathogenic variants and clinical characterisation of a representative cohort of Czech patients with RASopathies

**M. Nemcikova**<sup>1</sup>, **A. Baxova**<sup>2</sup>, **R. Beharka**<sup>3</sup>, **N. Dvorackova**<sup>4</sup>, **P. Fridrichova**<sup>5</sup>, **R. Gaillyova**<sup>3</sup>, **A. Gregorova**<sup>4</sup>, **M. Havlovicova**<sup>1</sup>, **T. Honzik**<sup>2</sup>, **V. Hurkova**<sup>4</sup>, **Z. Kalina**<sup>3</sup>, **J. Lastuvkova**<sup>7</sup>, **M. Malikova**<sup>1</sup>, **J. Nemeckova**<sup>3</sup>, **S. Prasilova**<sup>3</sup>, **A. Puchmajerova**<sup>1</sup>, **E. Seemanova**<sup>1</sup>, **M. Simandlova**<sup>3</sup>, **P. Solarova**<sup>2</sup>, **E. Silhanova**<sup>4</sup>, **J. Soukalova**<sup>3</sup>, **S. Vejvalkova**<sup>9</sup>, **M. Vlckova**<sup>1</sup>, **J. Vseticka**<sup>10</sup>, **A. Krepelova**<sup>1</sup>.

<sup>1</sup>Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and Motol University Hospital, Prague, Czech Republic, <sup>2</sup>Institute of Biology and Medical Genetics, Charles University 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic, <sup>3</sup>Department of Medical Genetics, Brno, Czech Republic, <sup>4</sup>Department of Medical Genetics, Ostrava, Czech Republic, <sup>5</sup>Department of Medical Genetics, Hradec Kralove, Czech Republic, <sup>6</sup>Prediko, Zlin, Czech Republic,

<sup>7</sup>Department of Medical Genetics, Usti nad Labem, Czech Republic, <sup>8</sup>Department of Paediatric Care, Prague, Czech Republic, <sup>9</sup>Department of Medical Genetics, Olomouc, Czech Republic, <sup>10</sup>Medical Genetics, Ostrava, Czech Republic.

Introduction: RASopathies represent a group of clinically and molecularly intersecting disorders comprising the Noonan syndrome (NS), NS with multiple lentigines (NSML), Noonan-like syndrome with loose anagen hair (NS/LAH), CBL-mutation associated syndrome, cardio-facio-cutaneous syndrome (CFCS), Costello syndrome (CS), neurofibromatosis type 1 (NF1) and Legius syndrome (LS). These clinical entities are due to pathogenic variants in RAS/MAPK signalling pathway genes. Overlap of phenotypic features makes clinical diagnosis challenging, while molecular genetic analysis fosters etiological diagnosis.

Materials and Methods: Targeted analysis of *PTPN11*, *SOS1*, *RAF1*, *RIT1*, *BRAF*, *KRAS*, *MAP2K1*, *MAP2K2*, *HRAS*, *SHOC2*, *CBL*, *RRAS*, *SPRED1* and *NF1* by Sanger -and/or HaloPlex enrichment assay (Agilent Technologies, USA) followed by next generation sequencing (MiSeq platform; Illumina, USA) in a representative group of 162 unrelated Czech cases with clinically diagnosed NS or related disorders, excluding NF1 and LS, was performed.

Results: Altogether, pathogenic variants were found in 69 (42.6%) of all cases and NS confirmed in 59 patients. Four patients were diagnosed with NSML, in 3 cases pathogenic variants indicating CFCS were detected and *HRAS* variants associated with CS were revealed in 2 instances. One patient was diagnosed with NS/LAH. All detected substitutions were missense, one variant in *RIT1* is novel.

Conclusion: Molecular, as well as clinical findings, generally correspond to previous studies. Our data facilitate genotype-phenotype correlations and aid clinical diagnosis in this heterogeneous group of disorders.

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#### P11.136

#### A family case of Mitchell-Riley syndrome/Martinez-Frias syndrome

**M. Sanchez-Gutierrez**<sup>1</sup>, **J. M. Carbonell-Perez**<sup>1</sup>, **J. Saenz-Hurtado**<sup>1</sup>, **E. Galan-Gomez**<sup>2</sup>, **R. Hernandez-Rastrollo**<sup>2</sup>;

<sup>1</sup>Hospital Infanta Cristina, Badajoz, Spain, <sup>2</sup>Hospital Materno Infantil, Badajoz, Spain.

Introduction: Mitchell-Riley syndrome/Martinez-Frias syndrome (mrs/mfs) is a rare, autosomal recessive disorder with multisystem involvement and poor prognosis. The Martinez-Frias syndrome is characterized by pancreatic hypoplasia, intestinal atresia, and gallbladder aplasia or hypoplasia, with or without tracheoesophageal fistula. When the patient also presents neonatal diabetes, it is called Mitchell/Riley syndrome.

Materials and methods: We report a family case of Mitchell-Riley syndrome/Martinez-Frias syndrome with a genetic mutation in RFX6. The patient is a healthy pregnant woman in her 26 weeks gestation (G3P2). Her first daughter, born of non-consanguineous gipsy parents, had a pancreatic hypoplasia, intestinal atresia and neonatal diabetes. She died at 17 months. This child was diagnosed of mrs/mfs and showed a homozygous missense mutation on exon 4, c.541C>T, p.R181W. The patient and her couple are heterozygous. Second daughter is heterozygous and showed at birth intestinal atresia that was surgically corrected. In last pregnancy, the fetus showed polyhydramnios and "double bubble" suggestive of duodenal atresia. The fetus showed the homozygous missense mutation, c.541C>T, and the pregnancy was terminated. The sister of the patient died at 15 month age, with neonatal dia-

betes and intestinal atresia. Her couple suffers diabetes type I and intestinal atresia. He has some relatives with intestinal atresia. Conclusions: Further study of patients with RFX6 mutations should clarify its role in pancreatic, intestinal and enteroendocrine cellular development. Furthermore, it should be investigated on the role of heterozygous mutation in the development of intestinal atresia and DM type I.

#### P11.137

##### De novo heterozygous mutation in ADGRL2 is associated with microlissencephaly and rhombencephalosynapsis

M. Vezain<sup>1,2</sup>, M. Lecuyer<sup>3</sup>, A. Laquerrière<sup>3,4</sup>, V. Dupé<sup>5</sup>, L. Pasquier<sup>6</sup>, L. Ratié<sup>5</sup>, V. David<sup>5</sup>, S. Odent<sup>5,6</sup>, L. Trestard<sup>7</sup>, T. Frébourg<sup>1,2,8</sup>, B. Gonzalez<sup>2</sup>, P. Saugier-Veber<sup>1,8,2</sup>,

<sup>1</sup>INSERM U1079, IRIB, Normandy University, Rouen, France, <sup>2</sup>Normandy Centre for Genomic Medicine and Personalized Medicine, Rouen, France, <sup>3</sup>ERI28, IRIB, Normandy University, Rouen, France, <sup>4</sup>Pathology Laboratory, Rouen University Hospital, Rouen, France, <sup>5</sup>UMR6290 CNRS IGDR, University of Rennes1, Faculty of Medicine, Rennes, France, <sup>6</sup>Department of Genetics, Rennes University Hospital, Rennes, France, <sup>7</sup>Department of Genetics, Belvédère Hospital, Mont-Saint-Aignan, France, <sup>8</sup>Department of Genetics, Rouen University Hospital, Rouen, France.

Rhombencephalosynapsis (RS) is a rare cerebellar malformation defined by vermician agenesis with fusion of the cerebellar hemispheres. In most cases, RS presents as a sporadic condition consistent with *de novo* dominant mutations. In sporadic forms, RS occurs in isolation or in combination with other cerebral and extra-cerebral malformations. The association of microlissencephaly with RS has never been reported so far. Moreover, the molecular bases of non-syndromic forms of RS remain unknown.

Using comparative index case-parents exome sequencing strategy, we identified a heterozygous *de novo* missense variant in the *ADGRL2* gene in a fetus affected with RS, microlissencephaly and growth retardation. *ADGRL2* encodes latrophilin 2, an adhesion G-Protein-Coupled Receptor whose exogenous ligand is  $\alpha$ -latrotoxin. *In situ* hybridization showed that *Adgrl2* was strongly expressed in the telencephalon, mesencephalon and rhombencephalon at HH12 for chicken embryo and E9.5 for mouse embryo. By using microfluorimetry experiments to evaluate the intracellular calcium ( $Ca^{2+}$ ) release in response to  $\alpha$ -latrotoxin binding, we showed that  $Ca^{2+}$  release was significantly reduced in the fetus amniocytes versus wild-type amniocytes from control fetuses at the same developing stage, as well as in HeLa cells transfected with mutant *ADGRL2* cDNA versus wild-type construct. A cell adhesion assay and a wound healing assay demonstrated that the mutation increased cell adhesion properties and reduced cell motility. HeLa cells overexpressing mutant *ADGRL2* displayed a highly developed cytoplasmic F-actin network.

Given the role of LAT-1, the *C. elegans* latrophilin orthologue, in the anterior-posterior tissue polarity pathway, we hypothesize that RS results from abnormal tissue polarity signaling.

#### P11.138

##### Analysis of mutations within the intron20 splice donor site of CREBBP, in patients with and without classical RSTS

H. G. Dauwelse<sup>1</sup>, M. J. van Belzen<sup>1</sup>, A. van Haeringen<sup>1</sup>, G. W. E. van Santen<sup>1</sup>, C. A. C. van de Lans<sup>1</sup>, E. Rahikkala<sup>2</sup>, L. Garavelli<sup>3</sup>, M. H. Breuning<sup>1</sup>, R. C. M. Hennekam<sup>4</sup>, D. J. M. Peters<sup>1</sup>, <sup>1</sup>LUMC, Leiden, Netherlands, <sup>2</sup>University of Oulu, Oulu, Finland, <sup>3</sup>Maria Nuova Hospital, Reggio Emilia, Italy, <sup>4</sup>University of Amsterdam, Amsterdam, Netherlands.

Whole exome sequencing of a patient with intellectual disability and without recognizable phenotype yielded a mutation, c.3779+1G>A, in the intron20 splice donor site of CREBBP. Mutations at different positions within the same intron20 splice donor site, were observed in 3 patients clinically suspected as having Rubinstein-Taybi syndrome (RSTS). All mutations were *de novo* and likely disease causing. To investigate a putative difference in splicing between the patient without RSTS phenotype and the three patients with RSTS phenotype, we analysed the effects of these mutations on splicing of the pre-mRNA of CREBBP. As no RNA of patients was available, we generated a new and improved exon-trap vector, pCDNAGHE, and tested the effect of the various mutations on splicing in vitro. All mutations lead to skipping of exon20. In one of the patients with an RSTS phenotype there was also some normal splicing detectable.

In conclusion, we describe a patient with a CREBBP mutation with intellectual disability and a phenotype not resembling RSTS. Three other patients in whom the diagnosis RSTS was clinically suspected, also carry a mutation in the same splice donor site. However, the splicing pattern obtained by exon trapping cannot explain the phenotypic differences and suggests the involvement of modifying genes, altering the expressivity of the disease phenotypes.

#### P11.139

##### A satellites 15q chromosome: clinical, cytogenetic and molecular characterization

S. Massara<sup>1</sup>, C. Ruggiero<sup>1</sup>, L. Espeche<sup>1</sup>, V. Cazayous<sup>2</sup>, V. Qualina<sup>2</sup>, L. Furfuro<sup>1</sup>, S. Rozental<sup>1</sup>, C. Sargiotti<sup>1</sup>,

<sup>1</sup>Centro Nacional de Genética Médica, Buenos Aires, Argentina, <sup>2</sup>Hospital El Cruce, Florencio Varela, Argentina.

Nucleolar organizer regions and satellites are located on the short arms of acrocentric chromosomes. There are several reports of satellites of non-acrocentric chromosomes. Sometimes, the ectopic NORs are attached to a terminal chromosome band as a result of balanced or unbalanced rearrangements that, depending on the particular type of rearrangement and the chromosomes involved, will be associated with phenotypic abnormalities or constitute rare familial polymorphisms.

Up to our knowledge, the present report describes the most distal 15q terminal deletion informed to date and the second associated with NOR translocation.

We report on a 21 years old patient with short stature and mild intellectual disability. The patient presented IUGR, hip dysplasia, feeding difficulties. Clinical assessment showed nail hypoplasia, widening halux, cubitus valgus and venous sinus corona radiata. She failed to complete first school.

The GTW (550 bands) banding technique showed a terminal deletion at 15q26.3 and NOR staining confirmed the presence of ectopic stalks. MLPA technique confirmed the subtelomeric deletion and arrayCGH analysis revealed that the anomaly involved a 4.6 Mb deletion. Parental karyotypes were normal.

It is difficult to establish a phenotype / genotype correlation due to the few reported cases of 15q terminal deletion. Pre and postnatal growth retardation seem to be a regular clinical feature and could be related to haploinsufficiency of the IGF1R gene. We emphasize the value of the right combination of cytogenetic and molecular techniques for the characterization of NORs ectopic regions.

#### P11.140

##### Somatic mosaic mutation in SETBP1 identified in the mother of a girl with Schinzel-Giedion syndrome

T. Vendrell Bayona<sup>1</sup>, P. Fernandez Alvarez<sup>2</sup>, A. Cueto<sup>1</sup>, F. Lopez<sup>1</sup>, M. Borregan<sup>1</sup>, L. Martinez<sup>1</sup>, M. Lines<sup>3</sup>, L. Carrasquilla<sup>4</sup>, E. Tizzano<sup>1</sup>,

<sup>1</sup>Department of Clinical and Molecular Genetics and Rare Diseases Unit, Hospital Vall d'Hebron, Barcelona, Spain, <sup>2</sup>Department of Clinical and Molecular Genetics and Rare Diseases Unit, Hospital Vall d'Hebron, Barcelona, Spain, <sup>3</sup>Barcelona, Spain, <sup>4</sup>Department of Pediatrics, Neonatology Section, Hospital Vall d'Hebron, Barcelona, Spain, <sup>5</sup>Endocrinology and Nutrition Department Arnau de Vilanova University Hospital, Barcelona, Spain.

Introduction: Schinzel-Giedion syndrome (SGS) is a severe congenital condition affecting many body systems. Manifestations include distinctive facial features, neurological problems, hydronephrosis, typical skeletal malformations, genital and cardiac anomalies. It is very rare, although the exact prevalence is unknown.

SGS is usually caused by *de novo* mutations in the SETBP1 (18q21.1), probably resulting in a gain-of-function or dominant-negative effect. Children with this condition have a higher risk of developing certain types of tumours including sacrococcygeal germ cell tumours.

We report on a girl with SGS caused by germinal mutations in the SETBP1 gene inherited from his mother who showed a somatic mosaicism.

Material and methods: The girl was diagnosed neonatally by her manifestations: coarse face with midface retraction, bilateral hydronephrosis, and skeletal anomalies. The mother was 34 year old with diabetes and a history of neonatal malignant sacrococcygeal teratoma. SETBP1 was analyzed by Sanger sequencing.

Results: The study revealed a heterozygous transition in SETBP1 resulting in a substitution at a highly conserved residue: c.2605A>C (p. Ser869Arg). The mutation was not found in other patients or in databases variants in the general population.

The mutation study in the mother showed different levels of somatic mosaicism ranging from 37% in blood and buccal cell samples to 6% in urine. Conclusions: SETBP1 is constitutionally mutated in developmental disorders and somatically mutated in cancer. We present the first molecularly documented evidence of germline and somatic mosaicism for a STBP1 mutation, identified in the mother of a child with Schinzel-Giedion syndrome.

## P11.141

### A novel splicing site mutation of PLK4 that is required for centriole biogenesis and genomic stability causes Seckel Syndrome.

T. Dinçer<sup>1</sup>, G. Budak<sup>1</sup>, C. Semerci<sup>1</sup>, A. Ölmez<sup>2</sup>, Y. Dodurga<sup>2</sup>, M. Özmet<sup>2</sup>, B. Toraman<sup>1</sup>, A. Yıldırım<sup>2</sup>, H. Ergin<sup>1</sup>, A. Alver<sup>2</sup>, N. Akarsu<sup>3</sup>, E. Kalay<sup>4</sup>;

<sup>1</sup>Department of Medical Biology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey, <sup>2</sup>Department of Medical Biology, Institute of Health Science, Karadeniz Technical University, Trabzon, Turkey, <sup>3</sup>Department of Medical Genetics, Pamukkale University Medical Faculty, Denizli, Turkey, <sup>4</sup>Department of Pediatric Neurology, Denizli State Hospital, Denizli, Turkey, <sup>5</sup>Department of Medical Biology, Pamukkale University Medical Faculty, Denizli, Turkey, <sup>6</sup>Department of Neonatology, Pamukkale University Medical Faculty, Denizli, Turkey, <sup>7</sup>Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey, <sup>8</sup>Gene Mapping Laboratory, Department of Medical Genetics, Hacettepe University Medical Faculty, Ankara, Turkey.

Seckel syndrome is a genetically heterogeneous rare autosomal recessive disorder characterized by severe prenatal and postnatal growth retardation, severe microcephaly with mental retardation and a characteristic 'bird-headed' facial appearance. Up to date, eight genes have been associated with Seckel syndrome of which their encoding protein participates in DNA damage response, genomic stability, centriole biogenesis and centrosome function such as mitotic spindle organization and mitotic progression. Using genome-wide SNP array genotyping and homozygosity mapping we mapped Seckel syndrome to chromosomal region 4q26.1-q26.3 in a Turkish family. Direct sequencing of PLK4 (Polo-Like Kinase 4) gene, which encodes a master regulator of the centriole biogenesis, revealed a homozygous splicing acceptor site transition resulting in hypomorphic premature translation termination mutation (p.Asp11Profs14Stop). PLK4-Seckel fibroblasts obtained from patient showed impaired centriole biogenesis and disrupted mitotic morphology. Moreover, G2/M delay and extended cell doubling time were observed. Further evaluation of the PLK4-Seckel cells showed that PLK4 is also essential for genomic stability and DNA damage response that are required for proper development of organism.

## P11.142

### Detection of low level somatic mosaic mutations in a gene panel for segmental overgrowth.

M. Alders, M. A. Haagmans, O. R. Mook, M. M. A. M. Mannens, H. J. Bliek;  
Department of Clinical Genetics, Academic Medical Center Amsterdam, Amsterdam, Netherlands.

Somatic mutations in genes of the PI3K/AKT/mTOR pathway causing segmental overgrowth may be present in very low percentages. This can hinder the identification of causal mutations. Sanger sequencing, but also standard NGS diagnostic pipelines, are able to identify mosaic mutations with a detection limit of approximately 10%. Targeted analysis of specific sites is most commonly used for a more sensitive detection of mutations. With the growing number of known causal mutations there is a need for a high sensitive mutation scanning method to analyze the complete coding sequences of the genes involved rather than analysis of hotspots. We have developed a NGS based gene panel test for segmental overgrowth (AKT1, AKT3, MTOR, PIK3CA, PIK3R2, PTEN, TSC1 and TSC2), especially for the detection of low level mosaicism. Samples are sequenced with high coverage (average 1500-2000 reads, minimum 500 reads) and an analysis pipeline was developed that is able to detect mosaic mutations as low as 1%.

## P11.143

### Clinical Evidence of Crossover in the SHOX Gene

E. Göktas, A. G. Zamanı, M. S. Yıldırım;  
Department of Medical Genetics, Meram Medical Faculty, Necmettin Erbakan University, KONYA, Turkey.

Mutations of the SHOX gene are common cause of various genetic growth disorders, including isolated short stature, L'eri-Weill syndrome and Langer syndrome. L'eri-Weill syndrome characterized by short stature, mesomelia and Madelung deformity.

We describe 10y old girl with disproportionate short stature and mesomelic shortening of the limbs. On X-ray examination shortening of the distal portion of the extremities and deformity in distal epiphysis of radius was seen. The patient was diagnosed L'eri-Weill syndrome together with the clinical findings and SHOX FISH analysis planned. The index patient's father, sister and paternal aunt showed disproportionate short stature and mesomelic shortening of the limbs but her brother was normal stature. Their chromosome analyses were normal but metaphase FISH with a SHOX-specific probe showed a deletion of the SHOX gene on the X chromosome in the female index patient, her sister and her paternal aunt but in the index patient's father FISH showed a SHOX deletion on the Y chromosome.

The deletion was originally located on the father's Y chromosome but transmitted to daughter's X chromosome by crossover during meiosis. The index patient's sister and paternal aunt have SHOX deletion on X chromosome. Interestingly, the index patient's brother FISH analysis was normal. This means that a crossover has occurred between the X and Y chromosomes in the father's spermatogenesis. After crossover, the father transmitted X chromosome with SHOX deletion to both daughters but transferred normal Y chromosome to son.

This family is presented as clinical evidence of crossover in the SHOX gene.

## P11.144

### Hepatoblastoma in a female with Simpson Golabi Behmel Syndrome: should we monitor females with SGBS as we do for males?

D. Haye<sup>1</sup>, E. Amouyal<sup>1</sup>, Y. Capri<sup>1</sup>, S. Rossignol<sup>2</sup>, C. Baumann<sup>1</sup>, A. Verloes<sup>1</sup>, L. Perrin<sup>1</sup>;

<sup>1</sup>Robert Debré Hospital, APHP, Paris, France, <sup>2</sup>Trousseau Hospital, APHP, Paris, France.

Simpson Golabi Behmel Syndrome (SGBS) (OMIM 312870) is a X-linked syndrome characterized by pre-and post-natal overgrowth, macrocephaly, characteristic facies, organomegaly, variable congenital malformations, mild/moderate intellectual deficiency and increased tumoral risk. It is caused by point mutations or genomic rearrangements in the glycan 3-gene (GPC3). We report on two girl sibs and their mother with SGBS. The first girl was born without macrosomia. She had a macroglossia that spontaneously regressed. She died from post-surgical complications of a hepatoblastoma at age three. Her sister was born after a pregnancy marked by polyhydramnios. She had macrosomia, macroglossia, nephromegaly and diffuse hepatomegaly. Wiedemann Beckwith syndrome was excluded. A novel c.213\_214insC heterozygous missense insertion (p.Q23TfsX46) was identified in exon 1 of GPC3 by Sanger sequencing in the family. X inactivation was not biased. The patient is currently followed on a yearly basis with abdominal ultrasound scan, and screened for tumoral markers and urinary catecholamins. The mother is asymptomatic.

Females with mutation in GPC3 can express SGBS. Dysmorphic features, macrosomia, diaphragmatic hernia, and developmental delay have been reported. Lyonisation has been proposed as the underlying mechanism for variable expression of SGBS in female patients (Yano 2011). SGBS predisposes to embryonal tumors. In males, the risk has been estimated at 8% (Cottetereau 2013). Cancers were only reported in two affected females: one low-grade ovarian carcinoma, and one breast cancer. Guidelines for neoplasia screening have been proposed for males. Observation of hepatoblastoma in females with SGBS raises the question of applying the same policies in both sexes.

## P11.145

### A de novo mutation in the SNAP25 gene in a patient with epileptic encephalopathy, hypotonia and contractures identified by trio-based exome sequencing

H. Gabriel<sup>1</sup>, S. Fehr<sup>1</sup>, P. Stoebe<sup>1</sup>, K. Hoertnagel<sup>1</sup>, S. Biskup<sup>1</sup>, C. Schell-Apacik<sup>2</sup>;

<sup>1</sup>CeGaT, Tuebingen, Germany, <sup>2</sup>Praxis f. Humangenetik, Berlin, Germany.

The Synaptosomal-associated protein-25 (SNAP25) is part of the SNARE protein complex which is involved in the release of neurotransmitters. So far, only two patients with mutations in SNAP25 have been described in the literature by whole exome sequencing; Rohena et al. (2013) identified a de novo SNAP25 mutation in an 15y old patient suffering from hypotonia, MR and epilepsy, and more recently, Shen et al. (2015) describe an 11y old patient who was suffering from MR, joint contractures, weakness and ataxic dysarthria. Exome sequencing revealed a de novo SNAP25 missense mutation. Here we describe a third patient with a de novo SNAP25 mutation. The patient was stiff at birth and had multiple joint contractures. First seizures were observed at the age of 2m. Epileptic encephalopathy was suggested after MRI imaging. He died at the age of 1y. As the result of the complex syndromal phenotype of the patient, trisomy 18 was suggested, however karyotyping and array-CGH testing showed a normal karyotype. Multiple genetic disorders were then suspected. Sequencing of a set of genes involved in these syndromes revealed no causative mutation. Finally, trio-exome sequencing was performed in an attempt to identify a genetic cause. We were able to identify a de novo SNAP25 mutation, p.Ile192Asn, which was predicted "pathogenic" by prediction tools. With this report we support the pathogenicity and demonstrate the clinical variability of SNAP25 gene mutations. Further, to our knowledge, this is the first patient combining the two major symptoms; contractures and epilepsy.

## P11.146

**Subnuclear re-localization of SOX10 and p54NRB correlates with a unique neurological phenotype associated with SOX10 missense mutations: genotype/phenotype correlations?**

S. Marlin<sup>1</sup>, A. Kavo<sup>2</sup>, S. Gherbi<sup>3</sup>, A. Chaoui<sup>2</sup>, A. Jacquette<sup>3</sup>, V. Baral<sup>2</sup>, R. Touraine<sup>4</sup>, Y. Watanabe<sup>2</sup>, L. Lecerf<sup>2</sup>, M. Holder<sup>5</sup>, R. Mendoza-Londono<sup>6</sup>, V. Pingault<sup>2</sup>, N. Bondurand<sup>2</sup>, <sup>1</sup>Centre de Référence des Surdités Génétiques, Département de Génétique, Paris, France, <sup>2</sup>INSERM, Creteil, France, <sup>3</sup>Service de Génétique, Hôpital Pitié-Salpêtrière, Paris, France, <sup>4</sup>Service de Génétique, CHU Saint Etienne, Saint Etienne, France, <sup>5</sup>Guy's et St Thomas's Hospital, London, United Kingdom, <sup>6</sup>The Hospital for Sick Children and University of Toronto, Toronto, ON, Canada.

SOX10 is a transcription factor with well-known functions in neural crest and oligodendrocyte development. Mutations in SOX10 were first associated with Waardenburg-Hirschsprung disease (WS4; deafness, pigmentation defects and intestinal aganglionosis). However, variable phenotypes that extend beyond the WS4 definition are now reported. The neurological phenotypes associated with some truncating mutations are suggested to be the result of escape from the nonsense-mediated mRNA decay pathway; but no mechanism was suggested for missense mutations, of which approximately 20 have now been reported. Here, we present two sporadic and one familial cases with mutations affecting the proline 175 of SOX10, presenting with WS2 (WS without HSCR) and an unusual peripheral and central progressive neurologic phenotype, including psychiatric diseases. Interestingly, these mutations, as well as one affecting the adjacent amino acid, all lead to specific subnuclear relocalization of the resulting proteins in vitro. Indeed, mentioned mutants co-localize in foci with the Drosophila behavior human splicing (DBHS) protein family members, including p54NRB, known as a paraspindle marker and new SOX10 partner. Of note, the co-transfection of wild-type and mutant SOX10 constructs led to the sequestration of wild-type protein in observed foci and altered synergistic activity between SOX10 and p54NRB. We propose that such dominant negative effect may contribute to or be at the origin of the unique progressive and severe neurological phenotype observed in affected patients. This new molecular/cellular mechanism could underlie the unique phenotype associated with some SOX10 missense mutations, and possibly other genes involved in related or unrelated pathologies.

## P11.147

**A Multigenerational Family with STAR Syndrome: A Novel FAM58A Variant and an Expansion of the Phenotypic Spectrum**

N. J. Boczek, T. Kruisselbrink, M. A. Cousin, P. R. Blackburn, E. W. Klee, R. H. Gavrilova, B. C. Lanpher; Mayo Clinic, Rochester, MN, United States.

Introduction: STAR syndrome is a rare X-linked disorder characterized by toe Syndactyly, Telecanthus, Anogenital malformations, and Renal malformations (OMIM: 300707). There are only ten cases described in the literature, all of which have loss-of-function variants in FAM58A. Seven of these cases have a de novo variant in FAM58A, while there are only two inherited mother-daughter pairs described.

Materials and Methods: The patient was delivered at term and was noted to have ductus arteriosus, bicuspid aortic valve, and bilateral 3-5 toe syndactyly at birth. She is currently 8 years of age and has a history of tethered cord, neurogenic bladder, learning differences, esotropia, and unilateral sensorineural hearing loss. The patient's mother and maternal half-sister have similar clinical histories with congenital heart defects, syndactyly, and anogenital malformations; however there are differences in phenotypic severity. Clinical whole exome sequencing (WES) was completed at Baylor Miraca Genetics to identify the genetic syndrome in this family.

Results: WES revealed a novel variant, c.651G>A (p.W217X), in FAM58A, identified in both the patient's mother and half-sister as well. This family represents the 11-13th cases described with STAR syndrome, and represents the third instance of familial inheritance.

Conclusions: To our knowledge, this is the first instance of a nonsense variant in FAM58A described in patients with STAR syndrome, and represents the third family with inherited disease. This family highlights that tethered cord and hearing loss should potentially be added to the phenotypic spectrum of STAR syndrome and shows the phenotypic variability of this syndrome amongst family members.

## P11.148

**A Further Family Of Stromme Syndrome Carrying CENPF Gene Mutation: Confirming The First Stromme Gene Description Study**

F. Ozkinay<sup>1</sup>, T. Atik<sup>1</sup>, E. Isik<sup>1</sup>, Z. Gormez<sup>2</sup>, M. Sagiroglu<sup>2</sup>, O. Atana Sahin<sup>3</sup>, N. Corduk<sup>4</sup>, H. Onay<sup>5</sup>; <sup>1</sup>Ege University, School of Medicine, Department of Pediatrics, Subdivision of Pediatric Genetics, Izmir, Turkey, <sup>2</sup>Informatics and Information Security Research Center, TUBITAK-BILGEM, Kocaeli, Turkey, <sup>3</sup>Acibadem University, Institute of Health Sciences, Biochemistry and Molecular Biology, Istanbul, Turkey, <sup>4</sup>Pamukkale University, Department of Pediatric Surgery, Denizli, Turkey, <sup>5</sup>Ege University, School of Medicine, Department of Medical Genetics, Izmir, Turkey.

**Background and Aims:** Stromme syndrome is an extremely rare genetic disorder characterized by microcephaly, anterior ocular chamber anomalies and "apple peel" type jejunal atresia. Here we report a Stromme Syndrome family with two affected siblings whose whole exome sequencing (WES) results revealed a homozygous truncating frameshifts mutation in CENPF gene. This family confirms very recent gene identification study reported by Filges et al.

**Case:** A 3-month-old girl was hospitalized due to prenatally diagnosed microcephaly, microphthalmia and dysmorphological features. Parents had a history of previous child with the same findings and 'apple peel' intestinal atresia as an additional finding.

Regarding the clinical features of both affected siblings, the diagnosis of Stromme syndrome was established. WES of these two cases showed the homozygous mutation (c.5912\_5913insA)/( p.T1974Nfs\*9) in CENPF gene(RefSeq NM\_016343.3; MIM#600235) as the most probable defect underlying the syndrome. Both parents were heterozygous for the mutation.

**Conclusions:** During our confirmation studies with another Stromme case, Filges et al. reported that CENPF gene is responsible for Stromme syndrome. This is the second report that the CENPF mutations cause Stromme syndrome.

## P11.149

**Unusual phenotypic features in the presentation of rare chromosome 6q subtelomeric microdeletion**

H. M. Kathom<sup>1</sup>, D. Avdjeva-Tzavella<sup>1</sup>, S. Bichev<sup>2</sup>, B. Karashova<sup>1</sup>, R. Tincheva<sup>1</sup>, A. Savov<sup>2</sup>; <sup>1</sup>Department of Clinical Genetics, University Pediatrics Hospital, Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>National genetics laboratory, Sofia, Bulgaria.

**Introduction:** Subtelomeric microdeletions are found to be major reason for psychomotor retardation and dysmorphism. Some such as 6q terminal microdeletion are very rare. Patients with this microdeletion present mainly with microcephaly, short neck, broad nose, psychomotor retardation, hypotonia and seizures, and retinal anomalies.

**Materials and Methods:** We report on a 9 years old girl, with unilateral choanal atresia, eye leucoma, facial dysmorphism, and muscular hypertonia at birth. Since 3 months of age she developed an epilepsy. At 9 years of age she presented with: severe mental retardation, short stature, microcephaly, low weight, low frontal and occipital hair lines, narrow forehead, downslanting palpebral fissures, "beak-like" nose, short, broad and slightly flattened philtrum, maxillary prognathism, high arched palate, low set ears, long neck with retroflexion, deformed chest, thoracolumbar scoliosis, long fingers and toes (arachnodactyly), and increased muscle tone. A screening for most common microdeletions, subtelomeric deletions and duplications, was performed using multiplex ligation-dependent probe amplification. Commercial MLPA kits (MRC-Holland) were used for detecting imbalance at the subtelomere regions of chromosomes; each kit consists of one probe for each subtelomere.

**Results:** A 6q terminal subtelomeric microdeletion was found, involving the PSMB1 gene.

**Conclusions:** In rare subtelomeric deletions is quite hard to establish clear phenotype-genotype correlation, as very small differences in the length of the affected region could change the spectrum of presented features. Our case is specific because of the lack of hypotonia, short neck, broad nose, retinal abnormalities, which are considered so far as major presentations of 6q subtelomeric deletion syndrome.

## P11.150

### Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy

*C. Mignot<sup>1,2,3</sup>, C. von Stülpnagel<sup>4,5</sup>, C. Nava<sup>6,8</sup>, D. Ville<sup>7</sup>, D. Sanlaville<sup>8,9,10</sup>, G. Lesca<sup>8,9,10</sup>, A. Rastetter<sup>6</sup>, B. Gachet<sup>6</sup>, Y. Marie<sup>6</sup>, C. Korenke<sup>11</sup>, I. Borggraefe<sup>12</sup>, D. Hoffmann-Zacharska<sup>13</sup>, E. Szczepaniak<sup>14</sup>, M. Rudzka-Dybala<sup>14</sup>, U. Yıldız<sup>15</sup>, H. Çağlayan<sup>16</sup>, A. Isapo<sup>17</sup>, I. Marey<sup>1</sup>, E. Panagiota<sup>18</sup>, C. Korf<sup>19</sup>, E. Rössier<sup>20</sup>, A. Ries<sup>21</sup>, S. Beck-Wolde<sup>21</sup>, A. Rauch<sup>22</sup>, C. Zwieier<sup>23</sup>, J. Hoyer<sup>23</sup>, A. Reis<sup>23</sup>, M. Mironov<sup>24</sup>, M. Bobylova<sup>24</sup>, K. Mukhin<sup>24</sup>, L. Hernandez-Hernandez<sup>25</sup>, B. Maher<sup>25</sup>, S. Sisodiya<sup>25</sup>, S. Wechuchyzen<sup>26</sup>, C. Myers<sup>27</sup>, H. Mefford<sup>27</sup>, K. Hörtnagel<sup>29</sup>, S. Biskup<sup>28</sup>, EuroEPINOMICS-RES MAE working group, J. Lemke<sup>29</sup>, D. Heron<sup>1,2,3</sup>, G. Kluger<sup>4,5</sup>, C. Depienne<sup>1,6</sup>;*

<sup>1</sup>APHP, Groupe hospitalier Pitié Salpêtrière Paris, Paris, France, <sup>2</sup>Centre de Référence „Déficiences Intellectuelles de Causes Rares“, Paris, France, <sup>3</sup>Groupe de Recherche Clinique (GRC) „Déficience Intellectuelle et Autisme“ UPMC, Paris, France, <sup>4</sup>Hospital for Neuropediatrics and Neurological Rehabilitation, Epilepsy Center for Children and Adolescents, Vogtareuth, Germany, <sup>5</sup>Paracelsus Medical University Salzburg, Salzburg, Austria, <sup>6</sup>Sorbonne Universités, UPMC Univ Paris 06, INSERM UMR S 1127, CNRS UMR 7225, ICM, Paris, France, <sup>7</sup>Service de Neurologie Pédiatrique, Hôpital Femme Mère Enfant, CHU de Lyon, Lyon, France, <sup>8</sup>Service de Génétique, Groupe Hospitalier Est, Hospices Civils de Lyon, Lyon, France, <sup>9</sup>Université Claude-Bernard Lyon 1, Villeurbanne, France, <sup>10</sup>CRNL, CNRS UMR 5292, INSERM U1028, Villeurbanne, France, <sup>11</sup>Klinikum Oldenburg, Zentrum für Kinder- und Jugendmedizin (Elisabeth Kinderkrankenhaus), Klinik für Neuropädiatrie u. angeborene Stoffwechselerkrankungen, Oldenburg, Germany, <sup>12</sup>Department of Pediatric Neurology and Developmental Medicine and Epilepsy Center, University of Munich, Munich, Germany, <sup>13</sup>Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland, <sup>14</sup>Clinic of Neurology of Child and Adolescents, Institute of Mother and Child, Warsaw, Poland, <sup>15</sup>Department of Pediatrics, Division of Child Neurology, School of Medicine, Dokuz Eylül University, Izmir, Turkey, <sup>16</sup>Boğaziçi University, Department of Molecular Biology and Genetics, Istanbul, Turkey, <sup>17</sup>AP-H

**Objective:** We aimed to delineate the neurodevelopmental spectrum associated with SYNGAP1 mutations and to investigate genotype-phenotype correlations.

**Methods:** We sequenced the exome or screened the exons of SYNGAP1 in a total of 251 patients with neurodevelopmental disorders. Molecular and clinical data from patients with SYNGAP1 mutations from other centers were also collected, focusing on developmental aspects and the associated epilepsy phenotype. A review of SYNGAP1 mutations published in the literature was also performed.

**Results:** We describe 17 unrelated affected individuals carrying 13 different novel loss-of-function SYNGAP1 mutations. Developmental delay was the first manifestation of SYNGAP1-related encephalopathy; intellectual disability became progressively obvious and was associated with autistic behaviors in eight patients. Hypotonia and unstable gait were frequent associated neurological features. With the exception of one patient who experienced a single seizure, all patients had epilepsy, characterized by falls or head drops due to tonic or myoclonic seizures, (myoclonic) absences, and/or eyelid myoclonia. Triggers of seizures were frequent (n=7). Seizures were pharmacoresistant in half of the patients. The severity of the epilepsy did not correlate with the presence of autistic features or with the severity of cognitive impairment. Mutations were distributed throughout the gene, but spared spliced 3' and 5' exons. Seizures in patients with mutations in exons 4-5 were more pharmacoresponsive than in patients with mutations in exons 8-15.

**Conclusion:** SYNGAP1 encephalopathy is characterized by early neurodevelopmental delay typically preceding the onset of a relatively recognizable epilepsy comprising generalized seizures (absences, myoclonic jerks) and frequent triggers.

## P11.151

### The new syndrome of hypogonadotropic hypogonadism, arrhythmogenic right ventricular dysplasia, facial dysmorphism and absence of corpus callosum is associated with TAX1 binding protein 3 gene variation

*M. Arafat<sup>1</sup>, E. Hershkovitz<sup>2</sup>, Z. Afawi<sup>3</sup>, N. Loewenthal<sup>2</sup>, A. Haim<sup>2</sup>, A. Levitas<sup>4</sup>, R. Parvari<sup>1,5</sup>;*  
<sup>1</sup>The Shraga Segal Department of Microbiology, Immunology & Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>2</sup>Pediatric Endocrinology & Metabolism Unit, Soroka University Medical Center, Beer Sheva, Israel, <sup>3</sup>Sackler Faculty of Medicine, Department of Neurology, Tel-Aviv University, Ramat Aviv, Israel, <sup>4</sup>Director of Pediatric Cardiac Imaging, Department of Pediatric Cardiology, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>5</sup>National Institute of Biotechnology Negev, Beer Sheva, Israel.

**Introduction:** Hypogonadotropic hypogonadism (HH) is a genetically heterogeneous syndrome characterized by decreased secretion or action of gonadotropin releasing hormone (GnRH), resulting in reduced sex hormone production and various clinical signs of hypogonadism. Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a cardiomyopathy disease that may result in arrhythmia, heart failure, and sudden cardiac

death. The aim of this study was to identify and characterize the genetic basis of congenital HH and ARVD/C in a consanguineous Bedouin family of nine members with two affected brothers.

**Materials and Methods:** Genotyping was done on the parents and two affected children. Exome sequencing was performed on the DNA of one brother. **Results:** The homozygous chromosomal regions contained 39 variations, reported in less than 10% of population variation databases. Following bioinformatics predictions for damaging effect, segregation in the family and analysis of prevalence in the Bedouin population, one candidate remained in the Tax1 (human T-cell leukemia virus type-I) binding protein-3 (TAX1BP3) gene. The variation- Ile33Thr substitutions is predicted to change the structure of PDZ domain that mediates protein-protein interactions and affected the stability.

**Conclusions :** The new clinical syndrome could be mediated by damaging the interaction of TAX1BP3 with the Potassium Channel KCNJ4 and improper function of the Wnt signaling pathway in which TAX1BP3 was suggested to participate.

## P11.152

### Temple syndrome with atypical features: a case report

*M. Yakoreva<sup>1,2</sup>, T. Kahre<sup>1,2</sup>, S. Pajusalu<sup>1,3</sup>, T. Reimand<sup>1,2</sup>, K. Öunap<sup>1,2</sup>;*

<sup>1</sup>Tartu University Hospital, United Laboratories, Department of Genetics, Tartu, Estonia, <sup>2</sup>Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia, <sup>3</sup>Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia.

Temple syndrome (TS) is a rare imprinting disorder characterized by pre- and postnatal growth retardation, hypotonia, poor feeding, motor and developmental delay, scoliosis, premature puberty and a variety of dysmorphic features. Maternal uniparental disomy of chromosome 14 is the underlying cause of TS. Isolated methylation defects and paternal deletions of imprinted locus 14q32 could be found in other TS cases.

We present a case of TS in a 4-years-old girl with some atypical features. The patient was born at the 35th weeks of gestation with weight 1832g (-1.5SD) and length 42cm (-2SD). Pregnancy was complicated by IUGR and oligohydramnios. Shortly after the birth AVSD, congestive heart insufficiency and kyphoscoliosis were diagnosed. During the first years of life, the child had feeding difficulties, poor weight gain, hypotonia and severe psychomotor developmental delay. Later, dysmorphic features, body asymmetry, increasing linear pigmentation on the arms and legs, hyperopia, strabismus and cognitive delay were noted. She started to walk independently at 2.5 years of age. Her height now is -5SD, weight and OFC -2.5SD.

UPD7-UPD14 MS-MLPA analysis revealed a complete hypomethylation of MEG3 gene (14q32.2). The same result was obtained using DNA from fibroblasts, urine and buccal swab. Comparative analysis of the SNPs using her mother's CMA results showed maternal heterodisomy of the whole chromosome 14. No mosaicism was detected.

Unlike previously described cases, our patient has more severe clinical presentation of TS with congenital heart defect, vision problems and skin pigmentation changes. This work was supported by the Estonian Research Council grant PUT355.

## P11.153

### Temple syndrome among patients with Prader Willi syndrome-like phenotype

*A. Lande<sup>1</sup>, M. Kroken<sup>1</sup>, K. Rabben<sup>2</sup>, L. Retterstøl<sup>1</sup>;*

<sup>1</sup>Dep of Medical Genetics, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Frambu Center for Rare Disease, Oslo, Norway.

**Introduction:** Temple syndrome (TS) and Prader Willi syndrome (PWS) share many features in infancy and childhood. TS is an important, but often neglected, differential diagnosis to PWS. We wanted to estimate the proportion of TS in patients with PWS-like phenotype.

**Materials and methods:** In all samples submitted for PWS testing during 2014 and 2015, we consecutively conducted analysis also for TS. A total of 150 samples were included. The main indications for testing were hypotonia in infancy or childhood, developmental delays and/or overweight. For TS-testing, we employed a methylation-sensitive MLPA-kit for detection of methylation aberrations in chromosomal region 14q32.

**Results:** 3 out of 150 patients (2%) with PWS-like phenotype proved to have TS. In comparison, 6 out of 150 patients (4%) got the diagnosis of PWS molecularly confirmed. All three TS patients fit the phenotype previously reported with the following shared features: low birth weight, neonatal hypotonia, feeding difficulties and slight developmental delay. The two oldest patients were overweight, and the oldest patient (9 years old) had learning disabilities. In one of the patients, we confirmed maternal uniparental di-

somy 14 by satellite testing. We will pursue such analysis in the other two patients.

Conclusions: TS is believed to be underdiagnosed, and our results support this view. In concordance with recent papers, we conclude that analysis for TS should be performed whenever genetic testing for PWS is indicated in children.

#### P11.154

#### Analysis of Polymorphisms and Haplotypes of NOS2, PTGS2 and VEGFA Genes in Individuals with Thalidomide Embryopathy

T. W. Kowalski<sup>1,2</sup>, L. Tovo-Rodrigues<sup>3,4</sup>, L. R. Fraga<sup>1</sup>, M. T. V. Sanseverino<sup>4,1,2</sup>, M. H. Hutz<sup>1</sup>, L. Schuler-Faccini<sup>1,2,4</sup>, F. S. L. Vianna<sup>1,2,4</sup>;

<sup>1</sup>Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, <sup>2</sup>Instituto Nacional de Genética Médica Populacional, Porto Alegre, Brazil, <sup>3</sup>Universidade Federal de Pelotas, Pelotas, Brazil, <sup>4</sup>Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Introduction: Thalidomide embryopathy (TE) affected more than 10,000 babies around the world in the 1960s. Thalidomide was a drug marketed as safe, once its teratogenic properties were unknown at the time. The molecular mechanisms underlying thalidomide's teratogenesis remain not fully comprehended. The hypothesis that teratogenesis occurs due to thalidomide's antiangiogenic properties has been largely investigated in experimental models. However, polymorphisms in genes affected by thalidomide were not evaluated in humans.

Methods: In the present study, ten functional polymorphisms in genes of angiogenesis pathway were accessed in 38 individuals with TE and 136 subjects without congenital anomalies of the Brazilian population. The Single Nucleotide Polymorphisms (SNPs) rs2779249 and rs2297518 of NOS2; rs689465, rs689466 and rs20417 of PTGS2; rs699947, rs1570360, rs2010963 and rs3025039 of VEGFA were genotyped by real-time PCR or Sanger sequencing. Microsatellite (CCTTT)n of NOS2 was accessed through fragment analysis. Haplotypes were inferred through Phase Bayesian algorithm. Statistical analysis was performed in SPSS v.18.

Results: All polymorphisms were in Hardy-Weinberg Equilibrium. It was not identified a significant difference of allelic, genotypic or haplotypic frequencies when comparing both groups evaluated. Risk haplotypes were compared with pattern of congenital anomalies in TE individuals, and also no significant association was observed.

Conclusion: In this investigation we could not suggest a risk or protective allele for TE in genes NOS2, PTGS2 and VEGFA, although other studies concerning endophenotypes and other candidate genes should also be performed in a larger sample of thalidomide's victims.

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#### P11.155

#### Spectrum of TSC1 and TSC2 alterations in Russian patients with tuberous sclerosis

E. N. Suspitsin<sup>1,2</sup>, G. A. Yanus<sup>1,2</sup>, M. Y. Dorofeyeva<sup>3</sup>, T. A. Ledashcheva<sup>4</sup>, E. N. Imyanitov<sup>1,2,5</sup>;

<sup>1</sup>St.-Petersburg State Pediatric Medical University, St.-Petersburg, Russian Federation,

<sup>2</sup>N.N. Petrov Institute of Oncology, St.-Petersburg, Russian Federation, <sup>3</sup>Research Institute of Pediatrics and Pediatric Surgery, Moscow, Russian Federation, <sup>4</sup>City Center for Medical Genetics, St.-Petersburg, Russian Federation, <sup>5</sup>I.I. Mechnikov North-Western Medical University, St.-Petersburg, Russian Federation.

Background: Tuberous sclerosis (TS) is a genetic disease characterized by development of hamartomas of multiple organs (brain, kidneys, retina, skin, etc.). TS prevalence in Russia approaches to 1:30000, however TSC1/2 mutation distribution has not been subjected yet to systematic studies.

Material and methods: DNA samples of 53 patients with clinical signs of tuberous sclerosis were tested for mutations in TSC1 and TSC2 genes using Sanger sequencing and MLPA. The vast majority of cases (50 of 53) were sporadic, i.e., no clinical signs of TS were detected in the parents.

Results: TSC1/2 mutations were detected in 46/53 patients (87%): 34 (74%) carried mutations in the TSC2 and 12 (26%) in the TSC1; this ratio is in agreement with data obtained in European countries and the USA. It is of note that mutations were detected in 5 of 7 patients with one major and 0-1 additional minor features. All but one alteration were unique; splice-site mutation TSC2 c.138+1G>A was found in 2 unrelated patients. Large rearrangements (exon deletions/duplications) were found in 5/46 (11%) and affected exclusively TSC2; this is substantially higher compared to estimates done in other studies (0.2-6%).

Conclusions: Tuberous sclerosis in Russian patients appears to have some peculiarities: 1) domination of sporadic form of the disease; 2) absence of recurrent mutations; 3) high frequency of large rearrangements.

Some patients with mutations had only one major diagnostic feature; thus, relaxed clinical criteria should be applied for justification of TSC1/2 mutation testing.

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#### P11.157

#### Enhancing the genetic understanding of Waardenburg Syndrome type II

S. Issa<sup>1,2,3</sup>, Y. Watanabe<sup>4</sup>, E. Faubert<sup>4</sup>, L. Lecerf<sup>4</sup>, N. Bondurand<sup>4</sup>, S. Marlin<sup>5</sup>, V. Pingault<sup>2</sup>;

<sup>1</sup>INSERM, U955, IMRB Team 6, Creteil, France, <sup>2</sup>INSERM, U1163, Institut Imagine, Paris, France, <sup>3</sup>Université Paris Est Creteil, Faculté de Médecine, Creteil, France, <sup>4</sup>AP-HP, Hôpital H.Mondor - Département de Génétique, Creteil, France, <sup>5</sup>Centre de Référence des Surdités Génétiques, Département de Génétique, Hôpital Necker, Paris, France.

Waardenburg syndrome (WS) is one of the most common types of autosomal dominant syndromic hearing loss and is characterized by association with pigment disturbances of hair, skin and iris. WS is clinically classified into four subtypes (WS1-4) depending on the presence of additional features. Mutations in 5 major genes of the neural crest pathway are found in this syndrome: MITF, PAX3, SOX10, EDNRB and its ligand EDN3. Nevertheless, a large number of cases still remain unexplained at the molecular level, notably in WS2 (70%). Whole Exome sequencing was performed in order to enhance the understanding of the molecular basis of WS2.

In a sporadic case (sequenced in trio), we were able to identify a missense variation in EDNRB, a gene previously involved in WS4, but with a different mode of transmission. Screening of our WS2 cohort led to the identification of 6 additional cases with mutations in this gene: 4 missense changes, one frameshift and one splice site mutation. Immunofluorescence experiments performed on the mutants and wildtype proteins (as well as previously described mutants and polymorphic missense changes as controls) revealed three mutants localizing in the cytoplasm instead of the membrane in different cell lines. A minigene was constructed for the splice site mutant and the resulting cDNA conserved 4 intronic base pairs, predicting a frameshift with early stop codon. In vitro functional tests are being conducted to analyze the transcriptional and post-translational regulation of key players in the melanocytic pathway upon over expression of each mutant.

#### P11.158

#### A clinical description of a rare familial case of Weaver syndrome caused by novel heterozygous EZH2 variant c.2050C>A (p.Arg684Ser)

A. Poluha<sup>1,2</sup>, K. Wojciechowska<sup>2</sup>, A. Bury<sup>3</sup>, A. Sobczynska-Tomaszewska<sup>4</sup>, K. Czerska<sup>4</sup>, I. Jaszcuk<sup>2</sup>, M. Holweg<sup>5</sup>, M. Lejman<sup>5</sup>, D. Winnicka<sup>5</sup>, M. Babicz<sup>2</sup>, B. Styka<sup>5</sup>, J. R. Kowalczyk<sup>2</sup>;

<sup>1</sup>Department of Pediatrics, Skaraborg Hospital, Skovde, Sweden, <sup>2</sup>Department of Pediatric Hematology, Oncology and Transplantology, Children's University Hospital, Lublin, Poland, <sup>3</sup>Department of Pediatric Endocrinology and Diabetology with Endocrine-Metabolic Laboratory, Children's University Hospital, Lublin, Poland,

<sup>4</sup>MedGen Medical Center, Warsaw, Poland, <sup>5</sup>Genetic Laboratory, Department of Pediatric Hematology, Oncology and Transplantology, Children's University Hospital, Lublin, Poland.

Weaver syndrome is a rare congenital disorder characterized by pre- and postnatal overgrowth, spectrum of developmental delay, advanced bone age and characteristic craniofacial morphology. It is caused by constitutional heterozygous EZH2 pathogenic variants.

The majority of cases thus far reported appear to have been sporadic and there is not much data available regarding long-term follow up of adults with EZH2-related Weaver syndrome or clinical variability in rare familial cases.

Here we present three individuals from a previously unreported family identified to have a novel EZH2 variant. Proband is a 7 years-old boy with developmental delay, moderate intellectual disability, behavioral problems, increased pre- and postnatal length, soft and doughy skin, broad thumbs, hipertrichosis and distinctive craniofacial dysmorphic features: macrocephaly, broad forehead, flat occiput, long philtrum, large ears, widely spaced eyes and horizontal chin crease. His 29 years-old mother and 55 years-old maternal grandmother are both of a tall stature and have similar craniofacial morphology although no evidence of intellectual disability.

Sequence analysis of EZH2 detected in a proband and then confirmed in mother and grandmother a novel, heterozygous variant c.2050C>A (p.Arg684Ser) in the highly conserved SET domain. It is likely to be pathogenic based on the fact that it segregates with the overgrowth phenotype in reported family and that recurrent substitution of the same amino acid residue Arg684 in EZH2 by a different amino acid is well known to be pathogenic.

In summary, we expand the mutational spectrum of EZH2-related overgrowth and point out to clinical variability in rare familial case.

## P11.159

### Molecular screening of EZH2: analysis of 23 patients with clinical features of Weaver Syndrome

**G. Gordo<sup>1</sup>, I. Dapía<sup>1</sup>, J. Tenorio<sup>1</sup>, P. Arias<sup>1</sup>, M. del Campo<sup>2</sup>, M. P. Tirado<sup>3</sup>, M. A. Ramos-Arrayo<sup>4</sup>, F. Santos-Simarro<sup>1</sup>, P. Lapunzina<sup>1</sup>;**

<sup>1</sup>Institute of Medical and Molecular Genetics-INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>Servicio de Genética, Hospital Vall D'hebron, Barcelona, Spain, <sup>3</sup>Servicio de Neurología pediátrico, Hospital La Paz, Madrid, Spain, <sup>4</sup>Servicio de genética. Hospital Virgen del camino, Pamplona, Spain.

**Introduction:** Weaver syndrome (WS) (MIM #277590) is an overgrowth disorder characterized by pre- and postnatal overgrowth, variable learning disability and distinctive craniofacial features. It is also characterized by advanced bone age (usually carpal osseous maturation), camptodactyly and an increased frequency of tumours. The prevalence of WS is still unknown due to the scant number of cases reported up to date. In 2011, mutations in the Enhancer of Zeste Homolog 2 (EZH2) were associated with the development of WS. EZH2 is a histone-methyltransferase that acts as the catalytic agent of the polycomb-repressive complex 2 (PRC2) and it is also involved in the PI3K/mTOR pathway which includes other genes implicated in different overgrowth disorders.

**Material and methods:** We performed molecular analysis of EZH2 in 23 patients with a clinical diagnosis of WS. Genomic DNA was extracted from peripheral blood, and mutational analysis by conventional sequencing was performed. Variants pathogenicity was evaluated through bioinformatics tools. **Results:** We found three different missense mutations affecting conserved domains of the protein. Two of them were not previously reported and are located in the SANT domain which is involved in DNA-binding. All three patients showed clinical features compatible with WS. To date, only one of them developed a tumour.

**Conclusions:** WS is a rare disease with few cases reported in the literature. In this study we described two new variants probably associated to the development of this disease. Additionally, one of the mutations appeared in a patient who developed a neuroblastoma early in the infancy.

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## P11.160

### Search of the genes responsible for Opitz C and Bohring-Opitz Syndromes

**R. Urreizti<sup>1</sup>, S. Mort-Farre<sup>1</sup>, N. Roca-Ayats<sup>1</sup>, H. Franco-Valls<sup>1</sup>, C. Esteve-Castañer<sup>1</sup>, J. Ponomarenko<sup>2</sup>, L. Cozzuto<sup>2</sup>, F. Munell<sup>3</sup>, A. M. Cueto<sup>3</sup>, E. F. Tizzano<sup>4</sup>, J. Dopazo<sup>4</sup>, B. Cormand<sup>4</sup>, L. Vilageliu<sup>1</sup>, J. M. Opitz<sup>5</sup>, G. Neri<sup>6</sup>, D. R. Grinberg<sup>7</sup>, S. Balcells<sup>1</sup>;**

<sup>1</sup>Universitat de Barcelona, IBUB, CIBERER, Barcelona, Spain, <sup>2</sup>Centre for Genomic Regulation, Barcelona, Spain, <sup>3</sup>Hospital Vall d'Hebron, Barcelona, Spain, <sup>4</sup>Centro Investigación Príncipe Felipe, BIER, CIBERER, Valencia, Spain, <sup>5</sup>University of Utah School of Medicine, Salt Lake City, UT, United States, <sup>6</sup>Università Cattolica Sacro Cuore, Rome, Italy.

Opitz C Syndrome (OTCS, MIM #211750) is a rare and severe pathology of unknown genetic cause and unclear pattern of inheritance. It presents with malformations and developmental delay and clinically overlaps with the more severe Bohring-Opitz Syndrome (BOS, MIM #605039). In BOS, half of the patients bear *de novo* truncating mutations in the ASXL1 gene.

We studied 14 families with patients diagnosed as OTCS or BOS, from different countries. ASXL1 was sequenced in all of them and 6 patients (and their parents) were analyzed by whole exome sequencing (WES).

We found an ASXL1 mutation in one Scottish BOS patient.

In a Spanish OTCS patient we found a *de novo* truncating mutation at the imprinted and maternally silenced MAGEL2 gene. The mutation was present on the paternal chromosome. Other truncating mutations in this gene have been found associated with the Shaa-Yang syndrome and with severe arthrogryposis, suggesting clinical heterogeneity for MAGEL2.

In an Italian OTCS patient we found a *de novo* mutation at a FOXP1 splice site, associated with developmental delay, speech impairment and contractures, all traits present in the patient.

Finally, two Russian OTCS sibs were reevaluated and re-diagnosed as affected with congenital myopathy and two recessive RYR1 mutations were identified by WES.

Selected variants in the exomes of the other three patients are currently being validated.

Our results suggest that OTCS is a highly genetically heterogeneous pathology.

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## P11.161

### Next generation sequencing: the prenatal diagnostic test for foetuses with severe ultrasound abnormalities?

**M. J. V. Hoffer<sup>1</sup>, A. C. J. Gijssbers<sup>1</sup>, S. Bollen<sup>1</sup>, M. E. Y. Laurensse-Bik<sup>1</sup>, I. J. H. M. van Minderhout<sup>1</sup>, S. Smit<sup>1</sup>, I. F. A. C. Fokkema<sup>2</sup>, A. Haeringen<sup>1</sup>, M. Koopmans<sup>1</sup>, T. P. Potjer<sup>1</sup>, K. van der Tuin<sup>1</sup>, G. W. E. Santen<sup>1</sup>, N. S. den Hollander<sup>1</sup>, C. A. Ruivenkamp<sup>1</sup>;**

<sup>1</sup>Dept. of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>2</sup>Dept. of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

At present, the standard prenatal genetic diagnostic screening tool for foetuses with ultrasound abnormalities is microarray analysis. However, recently, in patients with intellectual disability and/or developmental delay (ID/DD), next generation sequencing, proved to be a much better tool, it demonstrated in about 20% of the cases the disease-causing aberration. In our study we investigated whether whole exome sequencing (WES) could also be a promising tool to unravel the underlying defect for foetuses with ultrasound abnormalities.

Nine foetuses with a severe, unexplained ultrasound abnormality and their unaffected parents (trios) were postpartum included in our series of patients for WES. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including an ID/DD gene panel of ~700 genes for filtering of the data. Analysis of the whole exome was only performed when informed consent was available and the gene panel did not reveal a candidate mutation. All remaining variants with a potential clinical consequence were validated by Sanger sequencing. In 33% of the cases we were able to identify the causal genetic variant.

In conclusion, WES demonstrates to be a good diagnostic tool to unravel the underlying genetic cause in a substantial portion of the foetuses with severe ultrasound abnormalities.

## P11.162

### The process of genetic counselling and the genotype-phenotype correlation in a popliteal pterygium syndrome: a case report

**N. Capdevila, E. Gabau, R. Ballester, M. Guitart, N. Baena, S. Pina; Parc Taulí Sabadell, University Hospital, Sabadell, Spain.**

**Introduction:** Popliteal pterygium syndrome is a congenital malformation that includes orofacial, musculoskeletal and genitourinary anomalies. It is caused by mutations in the IRF6 gene and characterized by an autosomal dominant inheritance, incomplete penetrance and variable expression. Clinical manifestations may include from lower lip pits or mounds to cleft complete palate with the presence of cutaneous webbing across one or more major joints in the lower extremities.

**Presentation case:** Pregnant woman in the first trimester of pregnancy comes to genetic counselling consultation, because six months ago she had an abortion after detecting the presence of bilateral cleft lip with cleft palate and low mobility of the lower limbs in the foetus. After fetal autopsy, the diagnostic suspicion is a popliteal pterygium syndrome by the characteristic nail dysplasia. The IRF6 gene sequencing is performed, and Cys84Arg mutation is detected allowing the study of the carrier parents.

**Conclusion:** The joint assessment of the multidisciplinary team and the correct diagnostic orientation determined by the fetal autopsy has provided an accurate genetic diagnosis. Although the spectrum of mutations of IRF6 gene has an incomplete penetrance and variable expressivity, it has been possible to offer a correct and detailed genetic counselling to the progenitors on the current pregnancy.

## P12 Cancer genetics

### P12.001

#### Detection of a homozygous 11kb inversion by whole exome sequencing in a patient with Xeroderma pigmentosum

**P. Joset, S. Azzarello-Burri, T. Herget, M. Papic, M. Zweier, D. Niedrist, A. Baumer, A. Rauch;**

*Institute of Medical Genetics, University of Zurich, Schlieren-Zurich, Switzerland.*

Whole exome sequencing (WES) is a powerful tool in detecting point-mutations and small indels but is not commonly used to discover bigger structural rearrangements. With an overall diagnostic yield of about 50% in 262 single/trio cases, we found in approx. 70-80% of our cases a pathologic point substitution. Moreover, in about 11-16% we were detecting small deletions and in 9-14% small insertions. While the biggest pathologic deletion was 28 bps and the biggest pathologic insertion 13bps, structural rearrangements much bigger than those are difficult to detect, but might explain a certain percentage of our unsolved cases. Here we report a 20 year old male with spinocellular carcinoma of the face, lymphnode metastasis and already multiple facial tumorexisions. The initial Next Generation Sequencing ap-

proach with the Illumina cancer panel was negative for the suspicious genes DDB2, ERCC2, ERCC3, ERCC4, ERCC5, XPA und XPC. The subsequent targeted WES approach on additional genes associated with Xeroderma pigmentosum revealed an 11kb homozygous inversion with 1 breakpoint located within the coding exon 4 of the POLH gene. Mapping of the unmatched nucleotides form the reads resulted in a second intronic breakpoint 11189 bp downstream, between exon 8 and 9 of the POLH gene, predicted to disrupt the whole gene.

To our knowledge we report here for the first time a homozygous inversion detected by whole exome sequencing.

#### P12.002

#### The importance of Chromosome Microarray Analysis (CMA) in karyotypically normal children with Acute Lymphoblastic Leukemia (ALL)

*A. Mitrakos, A. Kattamis, M. Tzetzis, K. Katsibardi, E. Kanavakis, S. Kitsiou-Tzeli; University of Athens, Athens, Greece.*

The significance of genetic profiling in patients with ALL has been underlined by recent discoveries which have led to novel risk stratification subgroups and new treatment approaches, overall increasing Event Free Survival rates. Here we evaluate the efficiency of high resolution aCGH+SNP analysis when compared to conventional karyotype.

We studied bone marrow samples obtained after diagnosis from 8 children (median age 7 years old) with B-cell and B-cell precursor ALL (n=3 and n=5 respectively), that presented with normal or non diagnostic conventional karyotype and are part of a larger cohort. Following DNA isolation we utilized the high-resolution aCGH+SNP 2X400K platform (Agilent Technologies), that covers the entire genome with an average resolution of 7kb for CNVs, and can also identify regions of LOH. The results were compared to conventional cytogenetic techniques and assessed in the light of the clinical outcome for each patient.

Clinically relevant CNVs were detected in 7/8 cases (87.5%). The most common aberrations involved the ETV6 gene (n=5). One case with hyperdiploidy (54 chromosomes) and one case with hypodiploidy (37 chromosomes) were also detected, with the smallest CNV detected being a 28kb deletion involving PAX5 gene. Aberrant genomic regions harbored hematopoiesis (RUNX1), cell-cycle regulation (CDKN2A/2B), as well as oncogenes and tumor suppressor genes. Additionally, regions with LOH were identified, encompassing important genes (IKZF1, RARA).

We conclude that in a plethora of cases conventional cytogenetic techniques may not accurately depict the genetic profile of ALL patients in the clinical setting, increasing the necessity for additional approaches like CMA.

#### P12.005

#### Isochromosome der(17)(q10)t(15;17) in acute promyelocytic leukemia resulting in an additional copy of the RARA-PML and loss of the p53: report of two cases

*V. Djordjevic, M. Dencic-Fekete, J. Jovanovic; Clinical center of Serbia, Belgrade, Serbia.*

Introduction: Isochromosome of the long arm of the derivative chromosome 17, originating from the translocation t(15;17) [ider(17)(q10)t(15;17) or ider(17q)] in acute promyelocytic leukemia (APL), is a very rare chromosome aberration which has been associated with a poor prognosis.

Materials and Methods: We report two APL patients, their clinical and laboratory data associated with ider(17q). Cytogenetic analysis was performed on unstimulated bone marrow cells. Chromosomes were examined with modified Giemsa stain HG-banding technique. Interphase and metaphase fluorescence in situ hybridization (FISH) studies were performed on bone marrow cytogenetic specimens which were previously used for karyotype analysis. Detection of PML-RARA and RARA-PML fusion genes were performed using the DF SureFISH 15q24.1 together with the DF SureFISH 17q21.2 probes. Detection of p53 gene was performed using LSI TP53 (17p13.1) probe. DNA probes were applied following standard procedures outlined by the manufacturer. An RT-PCR assay was performed to detect the PML-RARA fusion gene.

Results: Cytogenetic analysis of bone marrow cells of both patients showed mosaic karyotype with the ider(17q) and reverse transcription polymerase chain reaction (RT-PCR) was positive for long (L) isoform of PML-RARA fusion transcript. FISH analysis confirmed extra copy of RARA-PML fusion gene or ider(17q) and loss of the normal tumor suppressor p53 gene in both of the patients.

Conclusions: Results both of the reported APL cases with ider(17q) indicated that the duplication of der(17) gives a growth advantage to the relevant clone which becomes dominant. Moreover, loss of the normal tumor suppressor gene p53 may also contribute to this growth advantage.

#### P12.006

#### Adenocarcinoma of the gastroesophageal junction by NF1: risk by offspring?

*N. Dragicevic<sup>1</sup>, A. Gehrig<sup>1</sup>, A. Rosenwald<sup>2</sup>, E. Kunstmenn<sup>1</sup>;*

*<sup>1</sup>Institute of Human Genetics, University of Würzburg, Würzburg, Germany, <sup>2</sup>Institute of Pathology, University of Würzburg, Würzburg, Germany.*

We report the occurrence and investigation of a adenocarcinoma of the gastroesophageal junction in a 41-year-old woman with previously clinical diagnosed neurofibromatosis type 1 in age of 20 year. A family history was positive, her father and uncle had café-au-lait spots. In the maternal family an aunt and cousin suffering from stomach and breast tumor.

The patient with multiple café-au-lait patches, axillary freckling and dermal neurofibroma, had also a benign tumor of the breast. She presented with loss of appetite, nausea, loss of weight, vomit, iron deficiency and respectively abdominal pain.

A gastroscopy with biopsy leading to the identification of a invasive moderately differentiated adenocarcinoma of the gastroesophageal junction Type of „Barrett- Carcinoma“.

By DNA sequencing in exon 26 of NF1 gene was detected germline deletion c.3457\_3460delCTCA. Genetic investigation of CDH1-gene was negative. To assess the relationship of the tumor to the NF1 mutation, DNA was extracted from paraffin-block and not revealed loss of heterozygosity at the NF1 gene. Son of the patient, 6 years old is the holder of the famous mutation in the NF1 gene. At was discussed about risks of morbidity and prevention of adenocarcinoma, as well as other possible mechanisms of tumorigenesis, excluding LOH (1, 2).

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#### P12.007

#### BRAF and KRAS mutations in colonic polyps as molecular marker of risk of metachronous advanced neoplasia.

*M. Juarez<sup>1</sup>, C. Egoavil<sup>1</sup>, E. Hernandez-Illan<sup>1</sup>, M. Rodriguez-Soler<sup>2</sup>, A. Garcia-Martinez<sup>1</sup>, P. Zapater<sup>3</sup>, C. Guarinos<sup>4</sup>, C. Alenda<sup>4</sup>, L. Castaño-Soler<sup>5</sup>, J. Martinez<sup>6</sup>, F. Ruiz<sup>6</sup>, L. Compañy<sup>7</sup>, J. Aparicio<sup>6</sup>, J. Casellas<sup>6</sup>, O. Murcia<sup>6</sup>, M. Giner-Calabuig<sup>1</sup>, A. Castillejo<sup>8</sup>, V. Barberá<sup>8</sup>, J. Soto<sup>9</sup>, R. Jover<sup>6</sup>;*

*<sup>1</sup>Unidad de investigación- Hospital General de Alicante, Alicante, Spain, <sup>2</sup>Unidad de Hepatología- Hospital General de Alicante, Alicante, Spain, <sup>3</sup>Servicio de Farmacología- Hospital General de Alicante, Alicante, Spain, <sup>4</sup>Servicio de Patología- Hospital General de Alicante, Alicante, Spain, <sup>5</sup>Hospital General de Alicante, Alicante, Spain, <sup>6</sup>Unidad de Gastroenterología- Hospital General de Alicante, Alicante, Spain, <sup>7</sup>Unidad de gastroenterología- Hospital General de Alicante, Alicante, Spain, <sup>8</sup>Unidad de Genética Molecular- Hospital General de Elche, Elche, Spain, <sup>9</sup>Unidad de gastroenterología- Hospital General de Alicante, Elche, Spain.*

BACKGROUND AND AIM: Our aim is to study if molecular characteristics of polyps can add information about risk of metachronous advanced neoplasia.

METHODS: We retrospectively included 308 patients diagnosed with colonic polyps (2007-2009) who have at least one surveillance colonoscopy made more than 6 months after the baseline, until 2014. 995 polyps were collected and tested for somatic BRAF(V600E) and KRAS(codons 12, 13) mutations. Patients were classified into 3 subgroups based on mutational profile of their polyps at baseline: 1) non-mutated polyps, 2) at least one BRAF-mutated polyp or 3) at least one KRAS-mutated polyp.

RESULTS: 661 polyps were classified as adenomas (66.4%), being 0.8% BRAF mutation and 11.6% KRAS mutation. 334 (33.6%) polyps were classified as serrated lesions: 281 (84.1%) HPs, 38 (11.4%) SSP, 8 (2.4%) TSA and 7 (2.1%) MP. A 39.4% of serrated polyps showed BRAF mutation, and 20.9% KRAS mutation. 289 patients could be classified in three mutational profiling groups: 14.9% were considered as BRAF mutated, 22.8% as KRAS mutated and 62.3% did not present any mutation in these markers. In univariate analysis, KRAS mutation was associated with development of metachronous advanced polyps (KRAS: 30.3%; BRAF: 16.3%; non-mutated: 15.6%; p = 0.029), more specifically advanced adenomas (KRAS 21.2%; BRAF 9.3%; non-mutated 10.0%; p=0.049). This association is also observed in the multivariate analysis, adjusted by age and sex (OR: 2.267, 95% CI: 1.152-4.461).

CONCLUSION: Our results suggest the presence of KRAS mutation in polyps at baseline is an independent risk factor for the development of metachronous advanced lesions.

## P12.008

### Risk factors for the presence of pathogenic APC and biallelic MUTYH mutations in patients with multiple adenomas

S. W. ten Broeke<sup>1</sup>, S. S. Badhal<sup>1</sup>, T. van Wezel<sup>2</sup>, H. Morreau<sup>2</sup>, F. J. Hes<sup>1</sup>, H. F. Vasen<sup>3</sup>, C. M. Tops<sup>1</sup>, M. Nielsen<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, Leiden, Netherlands, <sup>2</sup>Department of Pathology, Leiden, Netherlands, <sup>3</sup>Department of Gastroenterology and Netherlands Foundation for the Detection of Hereditary Tumours, Leiden, Netherlands.

**Background.** Patients with multiple colorectal adenomas may carry germ-line mutations in *APC* or *MUTYH*, but mutation detection rate seems to be declining. The aims of this study were (1) to assess the proportion of these mutations in patients with multiple adenomas and (2) to identify risk factors that predict mutation detection.

**Methods.** We performed mutation analysis of *APC* and/or *MUTYH* in a Dutch cohort of 1933 patients ascertained from family cancer clinics between 1992 and 2015. Risk factors were examined using (multinomial) logistic regression analyses.

**Results.** The overall detection rate declined from 54% before to 14% after 2004. The proportion of *APC/MUTYH* carriers in patients with <20 polyps was low (3.5%; 25/722). Only one mutation was identified in the patient group (n=198) of 10-19 adenomas diagnosed at age 60<. A younger age at adenoma diagnosis and a first degree relative (FDR) with polyps was associated with higher odds of finding an *APC* mutation, but CRC in a FDR was not. Having CRC was only predictive of finding biallelic *MUTYH* mutations.

	APC/MUTYH (n=332) <sup>3</sup> OR (95%CI)	APC (n=234)* OR (95%CI)	Biallelic MUTYH (n=95)* OR (95%CI)
<b>Adenoma count &lt;10</b>			
10-19	1.8 (0.79-4.2)	2.2 (0.5-9.3)	2.1 (0.8-6.1)
20-49	6.5 (3.6-12.0)	11.4 (3.9-32.8)	5.4 (2.5-11.6)
50-99	14.7 (7.3-29.5)	19.9 (6.2-63.4)	14.9 (6.2-34.6)
>100	77.6 (40.5-148.7)	202.79 (70.32-584.8)	20.3 (8.5-48.7)
<b>Age at adenoma diagnosis &gt;50</b>			
40-49	6.2 (3.7-10.0)	7.0 (3.8-12.0)	4.6 (2.3-9.2)
30-39	5.7 (3.3-9.7)	8.8 (4.7-16.5)	2.6 (1.1-6.1)
<30	7.7 (4.6-13.0)	15.0 (8.0-28.0)	1.3 (0.4-3.9)
No CRC			
CRC>50	0.74 (0.4-1.4)	0.54 (0.3-1.2)	3.8 (2.0-7.2)
CRC<50	0.55 (0.44-0.90)	0.67 (0.39-1.17)	4.3 (2.7-8.0)
<b>FDR polyps, no</b>			
Yes	1.7 (1.2-2.4)	2.1 (1.3-3.2)	1.2 (0.8-2.0)
<b>FDR CRC, no</b>			
Yes	0.79 (0.55-1.1)	0.77 (0.5-1.2)	0.9 (0.5-1.4)

**Discussion.** Adenoma count and younger age at adenoma detection are the main predictive factors of finding a mutation, but a FDR with CRC is not. For patients over age 60 with less than 20 adenomas testing does not seem justifiable. Our findings have an important impact on referral policy.

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## P12.009

### A dual role of sFRP3 modulating molecule in astrocytic brain tumors

A. Kafka<sup>1,2</sup>, D. Tomas<sup>3,4</sup>, L. Marković<sup>1</sup>, A. Varošanec<sup>1</sup>, N. Njirić<sup>1</sup>, Ž. Krsnik<sup>5</sup>, G. Mrak<sup>6</sup>, N. Pećina-Šlaus<sup>1,2</sup>;

<sup>1</sup>Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine University of Zagreb, Zagreb, Croatia, <sup>2</sup>Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia, <sup>3</sup>Department of Pathology, School of Medicine, University of Zagreb, Zagreb, Croatia, <sup>4</sup>University Hospital „Sisters of Charity“, Zagreb, Croatia, <sup>5</sup>Department of Neuroscience, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia, <sup>6</sup>Department of Neurosurgery University Clinical Hospital Zagreb, School of medicine, University of Zagreb, Zagreb, Croatia.

On a sample of 55 astrocytic brain tumors we determined the expression and localization of sFRP3 modulating molecule throughout different malignancy grades. Immunohistochemistry followed by digital scanning (Nano-Zoomer 2.0RS, Hamamatsu), and ImageJ (NIH, SAD) program were methods of choice for determining the levels of sFRP3 expression. Our results demonstrated that the differences between expression levels and malignancy grades were statistically significant. Moderate (P=0,014) and strong (P=0,028) nuclear expression levels were significantly different with pilocytic and diffuse astrocytomas showing higher expression values. When we divided our sample into two groups, both moderate and high cytoplasmic expression levels were significantly higher in glioblastomas than in the group comprising astrocytomas II and III. We also showed that high grade tumors had lower values of moderate (P=0,002) and strong nuclear (P=0,018) expression comparing to low grade tumors. Analysis of cytoplasmic staining showed that strong cytoplasmic expression was significantly higher in the astrocytomas III and glioblastoma group than in astrocytomas I and II group (P=0,048). Moreover, we found that lower grade astrocytomas had fewer membranous SFRP3 stain than higher grade astrocytomas and that this difference was significant (P=0,036). Our results demonstrated that SFRP3

protein expression levels decreased when located in the nucleus in higher astrocytoma grades, indicating expected behavior as an antagonist of Wnt signaling, whereas when located in the cytoplasm an increase in SFRP3 expression was noted in high grade as compared to low grade astrocytomas. This may suggest that SFRP3 can also act as an agonist of Wnt signaling promoting invasive behavior.

## P12.010

### Single nucleotide polymorphism (SNP) array analyses may help to development of personalized medicine in pediatric B-cell acute lymphoblastic leukemia

P. Carrasco Salas<sup>1</sup>, J. Nevado<sup>1</sup>, I. Vallcorba<sup>1</sup>, B. González<sup>2</sup>, D. Bueno<sup>2</sup>, L. Fernández<sup>3</sup>, M. Vela<sup>3</sup>, J. Valentín<sup>4</sup>, P. Lapunzina<sup>1</sup>, A. Pérez-Martínez<sup>2</sup>;

<sup>1</sup>INGEMM, Hospital Universitario La Paz, Madrid, Spain, <sup>2</sup>Unidad de Hemato-Oncología Pediátrica, Hospital Universitario La Paz, Madrid, Spain, <sup>3</sup>Centro Nacional de Investigaciones Oncológicas (CNIO), Grupo de Investigación clínica, Madrid, Spain,

<sup>4</sup>Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain.

**Introduction:** B-cell acute lymphoblastic leukemia (B-ALL), the most prevalent cancer in the pediatric population, is a malignant disease resulting from accumulation of genetic aberrations. Knowledge of these aberrations is useful for disease classification, prognosis, therapeutic purposes, and to provide an overall understanding of the pathogenesis of the B-ALL.

**Methods:** We retrospectively examined bone marrow samples from 32 pediatric B-cell ALL using the Illumina CytoSNP-850K BeadChip in the Illumina HiScan platform.

**Results:** Except for one, all patients showed copy number alterations (CNAs). Losses were more common than gains. Whole and partial copy neutral loss of heterozygosity (CN-LOH) were observed in 12 cases. Only four recurrent genetic alterations were found: hyperdiploidy (44% of the cases), deletion of CDKN2A/B genes (22%), deletion of PAX5 gene (16%) and deletion of ETV6 (9%) gene. Several possible target genes were identified, including SESN1, NME1 and BMPR1B, but additional studies are needed to confirm their implication in the disease. In a deceased high-risk patient, a partial heterozygous deletion of JAK2 gene were observed, which must be studied to confirm if it provides a gene expression signature similar to BCR-ABL1 pediatric ALL. These JAK-mutated cases have been proposed as logical targets for therapeutic intervention with JAK2 inhibitors.

**Conclusions:** SNP arrays are a powerful cytogenetic tool to define genetic abnormalities in B-ALL, including CN-LOH, a hidden chromosomal defect by standard methods. In addition, the use of this technology in clinical practice may help to develop individualized treatment plans for affected children.

## P12.011

### Genomic analysis identifies novel drivers and progression pathways in skin basal cell carcinoma

X. Bonilla<sup>1</sup>, L. Parmentier<sup>2</sup>, B. King<sup>3</sup>, F. Bezrukova<sup>4</sup>, G. Kaya<sup>5</sup>, V. Zoete<sup>6</sup>, V. B. Septyarskiy<sup>7,8,9</sup>, H. J. Sharpe<sup>10</sup>, T. A. McKee<sup>5</sup>, A. Letourneau<sup>11</sup>, P. G. Ribaux<sup>1</sup>, K. Popadin<sup>1</sup>, N. Basset-Seguin<sup>11</sup>, R. Ben Chaabene<sup>1</sup>, F. A. Santoni<sup>11</sup>, M. A. Andrianova<sup>7,8,9</sup>, M. Guipponi<sup>1</sup>, M. Garieri<sup>1</sup>, Verdan<sup>5</sup>, K. Grosdemange<sup>5</sup>, O. Sumara<sup>12</sup>, M. Eilers<sup>12</sup>, I. Afantis<sup>3</sup>, O. Michielin<sup>6,13</sup>, F. J. de Sauvage<sup>10</sup>, S. Antonarakis<sup>1,4,5</sup>, S. I. Nikolae<sup>1,2</sup>;

<sup>1</sup>University of Geneva, Geneva, Switzerland, <sup>2</sup>Hospital of Valais, Sierre, Switzerland, <sup>3</sup>NYU School of Medicine, New York, NY, United States, <sup>4</sup>University of Connecticut, Storrs, CT, United States, <sup>5</sup>University Hospitals of Geneva, Geneva, Switzerland, <sup>6</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland, <sup>7</sup>Institute of Information Transmission Problems, Moscow, Russian Federation, <sup>8</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation, <sup>9</sup>Lomonosov Moscow State University, Moscow, Russian Federation, <sup>10</sup>Genentech Inc., San Francisco, CA, United States, <sup>11</sup>University of Geneva, Paris, France, <sup>12</sup>University of Würzburg, Würzburg, Germany, <sup>13</sup>University of Lausanne and Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, <sup>14</sup>Institute of Genetics and Genomics of Geneva, Geneva, Switzerland.

Basal cell carcinoma of the skin (BCC) is the most common malignant neoplasm in humans. BCC is primarily driven by aberrant activation of the Sonic Hedgehog (Hh) pathway. However, its extensive phenotypical variation remains to be explained. The genetic profiling of 293 BCCs revealed the highest mutation rate observed in cancer (65 Mutations/Mb), with strong prevalence of UV-light signature mutations. 85% of BCCs harbored mutations in Hh pathway genes: mutually exclusive *PTCH1* (73%) and *SMO* (20%) ( $P=6.6 \times 10^{-6}$ ), *SUFU* (8%), and in *TP53* (61%). 85% of BCCs also harbored additional driver mutations in other genes implicated in BCC tumorigenesis. Recurrent driver mutations were observed in *MYCN* (30%), *PPP6C* (15%), *STK19* (10%), *LATS1* (8%), *ERBB2* (4%), *PIK3CA* (2%), *RAC1* (1%) and *N/K-H-RAS* (2%). Loss of function (LoF) and deleterious missense mutations were observed in *PTPN14* (23%), *RB1* (8%) and *FBXW7* (5%). In line with the mutational profiles detected by DNA sequencing, we observed activation of the Hh pathway as well as upregulation of target genes of the Hippo-YAP pathway and activation of *MYCN* target genes in RNAseq experiments.

The functional analysis of the novel tumorigenic driver mutations in *MYCN*, *PTPN14* and *LATS1* suggests their potential relevance in BCC tumorigenesis and provides an expanded molecular understanding of BCC.

#### P12.013

#### Clinical and genetic features of Birt-Hogg-Dubé syndrome in Spanish families

A. Lopez<sup>1</sup>, E. Llistosella<sup>2</sup>, E. Darder<sup>1</sup>, A. Velasco<sup>1</sup>, D. Sitjà<sup>2</sup>, A. Izquierdo<sup>1</sup>, E. Grau<sup>3</sup>, A. Solanes<sup>3</sup>, M. Navarro<sup>3</sup>, M. Salinas<sup>4</sup>, S. Iglesias<sup>4</sup>, À. Teulé<sup>4</sup>, G. Capellà<sup>4</sup>, C. Lázaro<sup>4</sup>, M. Pineda<sup>4</sup>, L. Leliubadaló<sup>4</sup>, J. Brunet<sup>1,4,5</sup>

<sup>1</sup>Hereditary Cancer Program, Catalan Institute of Oncology, Girona, Spain, <sup>2</sup>Dermatology Department, Josep Trueta University Hospital, Girona, Spain, <sup>3</sup>Hereditary Cancer Program, Catalan Institute of Oncology, Badalona, Spain, <sup>4</sup>Hereditary Cancer Program, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain.

**Introduction:** Pathogenic mutations in FLCN gene cause Birt-Hogg-Dubé syndrome (SBHD, OMIM #135150). It is inherited in a dominant manner and characterized by the presence of benign hamartomas of hair follicles (fibrofolliculomas and trichodiscomas), lung cysts, spontaneous pneumothorax and increased risk for renal neoplasm.

**Materials and Methods:** 17 families from our Hereditary Cancer Program were analyzed. Genetic testing identified FLCN deleterious mutation in 10 families. To date 30 patients were detected as carriers, all of them are under follow-up. Pathology reports were reviewed in order to classify skin lesions and renal neoplasms. Base line Thoracic CT scan to detect pulmonary bullae was indicated, and renal MRI was performed in all patients to screen for renal tumors.

**Results:** Focusing on carriers: 21 (70%) have coetaneous affection (15 have biopsied fibrofolliculomas/trichodiscomas); 8 (26%) developed pneumothorax and 7 were diagnosed of renal cancer (2 cromophob, 3 clear cell and 2 pending pathology results) and kidney sparing surgery could be performed for >3cm lesions. Only one patient shows the triad symptoms (skin, pulmonary and renal manifestations). Three carriers did not manifest any lesion, all of them were under 38 year old.

No previously reported mutations were identified in two families: c.573\_574delGAinsT and deletion of 1 and 2 exons. The other mutations were: c.1429C>T (nonsense), c.323G>T (missense), c.1733delC, c.3delG (frameshift) and c.1301-1G>A (splice site)

**Conclusions:** This data indicate that all carriers over 40 years old have clinical manifestation. Skin lesions appear as the first and more common manifestation. Screening for renal tumors allowed to avoid total nephrectomy.

#### P12.014

#### Differentially expressed long noncoding RNAs in bladder cancer

E. M. Borkowska<sup>1</sup>, S. Peter<sup>2</sup>, R. Dryton<sup>2</sup>, C. P. Rakshit<sup>2</sup>, A. P. Noon<sup>2</sup>, W. Chan<sup>1</sup>, J. W. F. Catto<sup>2</sup>

<sup>1</sup>Medical University of Lódz Department of Clinical Genetics, Lódz, Poland, <sup>2</sup>University of Sheffield Academic Urology Unit and Unit of Molecular Oncology, Sheffield, United Kingdom, <sup>3</sup>Max Delbrück Center of Molecular Medicine, Berlin, Germany.

**Introduction:** Bladder cancer (BC) is the fourth commonest male malignancy and one of the most expensive human cancers to manage. The majority of tumors are urothelial carcinoma in histologic type. Although many reports detail genetic events in urothelial cancer, alterations of epigenetic gene regulation are also important in this disease. Epigenetic gene regulation may occur directly or indirectly through noncoding RNA (ncRNA) species. To date, few data have reported the role of long noncoding RNA (lncRNA) in urothelial cancer and little is known of their function.

**Material and methods:** The expression of 17112 lncRNAs and 22074 mRNA was determined using microarrays in 83 normal and malignant urothelial samples (discovery step) and selected RNAs with qPCR in 138 samples (validation step). Significantly differentially expressed RNAs were identified and stratified according to tumour phenotype. siRNA knockdown, functional assays, and whole genome transcriptomic profiling were used to identify potential roles of selected RNAs.

**Results:** We observed upregulation of many lncRNAs in urothelial cancer that was distinct to corresponding, more balanced changes for mRNAs. In general, lncRNA expression reflected disease phenotype. We identified 32 lncRNA with potential roles in disease progression. Focusing upon a promising candidate, we implicate upregulation of AB074278 in apoptosis avoidance and maintenance of a proliferative state in cancer through a potential interaction with EMP1, a tumour suppressor and a negative regulator of cell proliferation.

**Conclusion:** We have identified many lncRNAs significantly altered in urothelial cancer and associated with disease progression and tumor subtypes.

#### P12.015

#### The expression of PIEZO1 and PIEZO2 ion channels in human and mouse bladder carcinoma

E. O. Etem<sup>1</sup>, G. G. Ceylan<sup>2</sup>, S. Ozaydin<sup>3</sup>, C. Ceylan<sup>3</sup>, T. Kuloglu<sup>4</sup>, I. Ozercan<sup>5</sup>

<sup>1</sup>Firat University Medical School Department of Medical Biology, Elazığ, Turkey,

<sup>2</sup>Yildirim Beyazit University Medical School Department of Medical Genetics, Ankara, Turkey, <sup>3</sup>Turkey yüksek ihtisas education and research hospital department of urology, Ankara, Turkey, <sup>4</sup>Firat University Medical School Department of Histology, Elazığ, Turkey, <sup>5</sup>Firat University Medical School Department of Pathology, Elazığ, Turkey.

**Introduction:** Action and pressure-sensitive PIEZO ion channels, express in bladder, are cation-selective mechanosensitive channels. PIEZO1 is a MSC protein and it is encoded by PIEZO1 gene in humans. PIEZO2 is a close homolog of PIEZO1. We aim to evaluate PIEZO1/PIEZO2 expression in postnatal period (P0-1) in mice bladder tissue as developmental and bladder cancer (BCa) tissue of mouse and human.

**Material/Methods:** The detection of developmental expression was performed in P0,P7,P14,P21,P28,P36 and P90 in bladder tissue of BalbC strain mice. Mice were divided into BCa group(n=40) and control group(n=10). BCa in mice was created by using N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). A total of 50 subjects were included in the study (40=BCa patients, 10=controls). After histopathological evaluation for BCa, expression of PIEZO1/2 genes were examined by RT-PCR and immunohistochemistry in tumor and normal tissues.

**Results:** PIEZO1 expression increased 21th and 90th days whereas PIEZO2 expression increased 7th day and decreased 90th day as developmentally. It was found in situ carcinoma in 14 samples, adenocarcinoma in 6 samples and benign proliferative changes in 10 samples in mice. Comparing with control group, it was detected significantly increase in expression of PIEZO1/2 in cancer groups in human and mice. Immunoreactivity had been observed against PIEZO1/2 both human and mouse bladder tissue with normal or cancer.

**Conclusions:** The developmental changes in specific days of PIEZO expression is demonstrated to play role in BCa development. Although roles of PIEZO1/2 ion channels in BCa isn't known exactly, the dysfunction of PIEZO1/2 ion channels expression may contribute the carcinogenesis of BCa by causing proliferative changes.

#### P12.016

#### Genetic and epigenetic profile of tumor cells of 126 patients with diffuse brain gliomas

Z. Zemanová<sup>1</sup>, H. Lhotská<sup>1</sup>, K. Svobodová<sup>1</sup>, H. Čechová<sup>2</sup>, S. Ransdorfová<sup>2</sup>, L. Lízová<sup>1</sup>, F. Kramar<sup>3</sup>, Z. Krejčík<sup>2</sup>, L. Pavlistová<sup>1</sup>, S. Izáková<sup>1</sup>, A. Berková<sup>1</sup>, K. Skipalová<sup>1</sup>, K. Michalová<sup>1</sup>

<sup>1</sup>Center of Oncocytogenetics, Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic, <sup>2</sup>Institute of Hematology and Blood Transfusion, Prague, Czech Republic, <sup>3</sup>Department of Neurosurgery, Central Military Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic.

Diffuse gliomas are the most common brain tumors and comprise astrocytomas, oligodendrogliomas, and oligoastrocytomas (WHO grades II-III), and glioblastomas (grade IV). Lower-grade gliomas (LGG, grade II-III) have highly variable clinical behavior that is not adequately predicted on the basis of histological classification. Assessment of the genome aberrations will probably allow more robust and prognostically relevant classification of these tumors.

We performed genome-wide analyses of 126 samples of gliomas (100x LGG, 26x glioblastoma) with the aim to investigate the genetic and epigenetic background of different tumor subtypes. The tissues taken from primary tumors were analyzed using I-FISH with VYSIS probes (Abbott), SNP array (Illumina), and MLPA (MRC-Holland) to detect recurrent chromosomal aberrations, copy number variations, IDH mutations and hypermethylation of MGMT and MMR genes promoters.

Mutation of IDH genes was detected in 75.0% LGG. A total of 41.3% LGG patients with mutated IDH had simultaneous co-deletion 1p/19q, which is considered the indicator of better response to radio-chemotherapy. Acquired UPD17p was proven in 65.0% of astrocytomas and 23.1% of glioblastomas. In 46.2% glioblastomas, shattering of different chromosomes (chromothripsis) always in combination with complex changes was observed. In 39.0% LGG, new recurrent finding - hypermethylation of MLH3 gene promoter was detected. In our study, patients with LGG and hypermethylated MLH3 had significantly longer OS (p=0.001).

In conclusion, molecular-cytogenetic diagnostics is a powerful tool to obtain prognostically relevant information in glioma patients and therefore should be an integral part of examination in patients with brain tumors.

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**P12.017****Hereditary breast/ovarian cancer: a systematic screening of 94 cancer associated genes in 500 consecutive index cases****A. Gehrig, I. Schmitt, C. R. Müller;***Department of Human Genetics, Würzburg, Germany.*

**Introduction:** In about 25% of cases, hereditary breast and ovarian cancer (HBOC) is caused by mutations in BRCA1 or BRCA2. Additional DNA repair genes such as CHEK2, ATM, PALB2, RAD51C and others have been implicated in HBOC.

**Methods:** All 500 patients fulfilled the inclusion criteria defined by the German HBOC Consortium. After target enrichment with the TruSight cancer panel™ (Illumina) designed to simultaneously analyse 94 cancer associated genes - sequencing was performed on a MiSeq (Illumina). Variant analysis was done by GensearchNGS software (PhenoSystems) and the NextGENe CNV detection tool (Softgenetics).

**Results:** Coverage was at least 50-fold across 93% of all investigated coding regions. We focused on protein truncating mutations since functional data on other variants in these genes are scarce. In addition to BRCA1 and BRCA2, we detected pathogenic mutations (class 4 and 5) in the genes: ATM, CHEK2, NBN, PALB2, RAD51D, BRIP1, TP53, PTEN, CDH1, MSH6, FANCA, FANCC, FANCI, FANCM, XPC, ERCC2, RECQL4, SLX4, WRN, BLM, BUB1B, PMS1 and SBDS. CNVs were found in BRCA1, BRCA2, NBN and WRN.

**Conclusions:** We identified monoallelic, likely pathogenic mutations in DNA repair genes other than BRCA1/2 in about 10% of HBOC cases. However, the causative association to HBOC and the prospective tumor risks for many of these mutations and genes have yet to be determined. Of note, extending the analysis to a larger number of genes proportionally increases the number of variants of uncertain significance and the workload to survey and classify them.

**P12.018****TruSight Enrichment Workflow vs PCR Amplicon Workflow (MASTR) for targeting cancer genes****M. Chatzidaki<sup>1</sup>, Y. L. Loukas<sup>1</sup>, G. Thodi<sup>2</sup>, E. Molou<sup>2</sup>, Y. Dotsikas<sup>1</sup>, K. Schoulipi<sup>2</sup>, O.***Triantafylli<sup>2</sup>,*<sup>1</sup>*Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, <sup>2</sup>Neoscreen LTD, Athens, Greece, <sup>3</sup>Department of Mental Health and Social Welfare, Hospital Paidon "Agia Sofia", Athens, Greece.*

**Introduction:** Breast cancer has been associated with mutations in other low and high penetrant genes except of BRCA. This knowledge allow us to identify more women at risk for breast cancer. The purpose of this study was to compare PCR Amplicon Workflow (BRCA HC MASTR Plus) which detects mutations and CNVs in 26 "breast cancer" genes and TruSight Enrichment Workflow (TruSight cancer) which targets 94 "cancer" genes.

**Materials/Methods:** 30 patients with mild to severe family history were subjected to BRCA HC Plus panel and 12 to the TruSight cancer panel (TC). Three runs were conducted using MiSeq 600V3 reagent kit in the former case, i.e. 10 samples per run, while 4 samples each time were loaded on a MiSeq 300V2 kit, regarding the latter panel.

**Results:** Both panels require a low input of DNA and have an efficient workflow. Regarding hands- on time, it seems that BRCA HC MASTR Plus workflow is shorter and it may be easier for a less experienced user. Considering the quality of the final results, a high Q-score was achieved with both panels. However, the average coverage (read-depth) reached with the former panel was higher i.e. about 500x for most of the targeted genes compared to the one reached with the TC, regarding the common cancer genes.

**Conclusion:** TC panel could be a solution for cases with a rather inconclusive family history, but for patients with a distinct phenotype BRCA HC MASTR plus panel offers a rather more robust and efficient method for confirming diagnosis.

**P12.019****BRCA1 and BRCA2 mutation spectrum in Lebanese women undergoing testing for hereditary breast/ovarian cancer****R. Badra<sup>1</sup>, J. Abbas<sup>2</sup>, M. Assaad<sup>1</sup>, F. Boulos<sup>1</sup>, N. El Saghier<sup>3</sup>, C. Mounsef<sup>1</sup>, D. Mukherji<sup>2</sup>, Z. Salem<sup>2</sup>, M. Seoud<sup>3</sup>, C. Farra<sup>1</sup>;**<sup>1</sup>*Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon, <sup>2</sup>Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon, <sup>3</sup>Department of Obstetrics and Gynecology, American University of Beirut Medical Center, Beirut, Lebanon.*

**Introduction:** In Lebanon, breast cancer is considered the most common malignancy among women. Up to 10% of breast cancers are directly related to germline mutations in BRCA1 or BRCA2 genes. In this retrospective study, we aimed at reporting the prevalence of BRCA mutations in a Lebanese cohort of individuals with personal or strong family history of breast/ovarian cancer, in order to determine the role of BRCA testing in breast cancer risk assessment.

**Materials and Methods:** Genetic results of all Lebanese patients, who underwent full sequencing of BRCA1/2 at AUBMC between 2011 and 2015, were reviewed. Demographic and personal/family cancer history data were collected.

**Results:** Deleterious mutations were identified in 10.6% of the families (20/188) (16BRCA1, 4BRCA2). In total, twelve deleterious mutations were recognized, four of them were novel. In 30% (6/20) of the carrier families, the BRCA1 C44F mutation was identified; this is reportedly the most common mutation in our population, suggesting a possible founder effect. Two other mutations BRCA2 IVS24-1G>A and BRCA1 E720X accounted for respectively 15% and 10% of the familial mutations. Twenty five variants were considered of unknown clinical significance (10 BRCA1, 15 BRCA2).

**Conclusions:** Prevalence of BRCA mutations was found to be high among tested women. Three mutations accounted for more than 55% of the total detected mutations. This study stresses on the importance of BRCA testing in Lebanese women at high risk for breast/ovarian cancer. It also highlights interesting data in terms of prevalence and spectrum of BRCA mutations in our population.

Funding source: MPP/AUB

**P12.020****Prevalence and reclassification of BRCA1 and BRCA2 variants of uncertain significance in Spanish breast cancer families****A. Gisbert<sup>1</sup>, M. Cornet<sup>1</sup>, D. Fisas<sup>2</sup>, C. López<sup>2</sup>, E. Martínez<sup>1</sup>, M. Pintor<sup>2</sup>, N. Calvo<sup>2</sup>, T. Ramón y Cajal<sup>2</sup>, A. Lasa<sup>1,2</sup>;**<sup>1</sup>*Genetics Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>2</sup>Oncology Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>U-705, CIBERER, Barcelona, Spain.*

**INTRODUCTION:** In the last two decades, the molecular diagnosis of hereditary breast and/or ovarian cancer has been based on the identification of germline inactivating mutations within the BRCA genes. Unfortunately, variants of uncertain significance (VUS) still occur in 5-10 % of tests. The aim of this study was to reassess the pathogenicity of VUS identified in families counseled at our hospital.

**MATERIAL AND METHODS:** VUS were reclassified into class I, II, III, IV and V following the International Agency for Cancer Research (IARC) recommendations. This was done by means of a thorough review of online database resources such as BIC (<http://research.ncbi.nlm.nih.gov/bic/>), LOVD (<http://www.lovd.nl/3.0/home>) and KConFab (<http://www.kconfab.org/Progress/Mutations.aspx>) together with a systematic literature review. The final classification was based on the use of integrated analyses, in vitro transcript assays and in silico tools.

**RESULTS:** From 1995 to 2015, 1200 families were tested for BRCA1/2 germline mutations. A total of 307 variants were identified in 253 families and 182 were unique.

Our integrative approach allowed us to reclassify 96/182 variants originally categorized as VUS (class III). Eighty of them (83%) were predicted to be non-pathogenic or class I, 7 (7%) were reclassified as class II, 5 (5%) as class IV and 4 (4%) as class V. The other 86 remained as class III.

**CONCLUSIONS:** The continuous reassessment of VUS remains a challenge for clinicians and geneticists. The wealth of information provided by NGS studies, facilitated the reclassification of more than half of our original VUS, thus allowing us to improve our preventive strategy.

**P12.021****Reliable detection of BRCA-mutations in formalin-fixed, paraffin-embedded ovarian carcinomas using smMIP-based next generation sequencing****R. Weren<sup>1</sup>, A. Mensenkamp<sup>1</sup>, M. Simons<sup>1</sup>, A. Sie<sup>1</sup>, M. Nelen<sup>1</sup>, H. Ouchene<sup>1</sup>, M. van Asseldonk<sup>1</sup>, A. Eijkelenboom<sup>1</sup>, J. Shendure<sup>2</sup>, A. Hoischen<sup>1</sup>, B. Tops<sup>1</sup>, N. Hoogerbrugge<sup>1</sup>, M. Ligtenberg<sup>1</sup>;**<sup>1</sup>*Radboudumc Nijmegen, Nijmegen, Netherlands, <sup>2</sup>University of Washington, Seattle, WA, United States.*

**Introduction.** Poly(ADP-ribose) polymerase (PARP) inhibitors are promising novel therapies for ovarian cancer patients who have developed BRCA1 or BRCA2 mutation-positive tumors. Approximately 15% and 8% of these patients have an inactivating germline or somatic BRCA-mutation, respectively. Therefore, assessing the BRCA-mutation status in DNA derived from ovarian carcinomas is crucial to select patients who may benefit from these treatments. However, the detection of germline and somatic mutations in BRCA1 and BCRA2 is hampered by the poor quality of DNA derived from formalin-fixed, paraffin-embedded (FFPE) ovarian carcinomas and the complexity of both genes.

**Materials and Methods.** We present a single molecule molecular inversion probe-based targeted sequencing approach to detect germline and somatic

mutations in BRCA1 and BRCA2 in DNA derived from FFPE ovarian carcinomas. This method was applied to 107 ovarian carcinomas derived from patients with a BRCA1 (n=24) or BRCA2 (n=14) germline mutation and sporadic patients (n=50) before and after chemotherapy. Calculation of the technical sensitivity and reduction of the levels of sequencing background noise was achieved using molecular barcoding to recognize unique template molecules.

**Results.** All germline mutations in BRCA1 and BRCA2 were successfully detected. In addition, pathogenic somatic mutations in BRCA1 and BRCA2 were observed in five sporadic patients (10%).

**Conclusions.** Our approach enables the rapid and reliable detection of both germline and somatic mutations in BRCA1 and BRCA2 in DNA derived from FFPE ovarian carcinomas. Consequently, this method can be applied to select ovarian cancer patients who may benefit from treatments with PARP inhibitors and genetic counseling.

#### P12.022

#### Experience of BRCA clinical testing for 3,200 patients: An international perspective

*L. Wange<sup>1</sup>, S. Wehnelt<sup>1</sup>, D. Gebs<sup>2</sup>, J. House<sup>2</sup>, K. Copeland<sup>3</sup>, L. Esterling<sup>2</sup>, S. Manley<sup>2</sup>, H. C. Cox<sup>2</sup>,*

<sup>1</sup>Myriad GmbH, Munich, Germany, <sup>2</sup>Myriad Genetic Laboratories, Inc., Salt Lake City, UT, United States, <sup>3</sup>Myriad Genetics GmbH, Zurich, Switzerland.

**Introduction:** Inherited mutations in the BRCA1 and BRCA2 genes are the most common cause of hereditary breast and ovarian cancer (HBOC) across human populations. Clinical screening of germline variants has been routine for over 2 decades in countries such as the USA. However, testing is becoming increasingly accessible on the global scale. We report the outcomes of clinical BRCA genetic testing in a cohort ascertained across 33 countries representing 6 continents.

**Methods:** This study assessed 3,229 consecutive individuals undergoing BRCA1 and BRCA2 full sequencing and large rearrangement analysis in a DAkkS accredited laboratory in Munich, Germany from 2013 to 2015. Clinical information was obtained from test requisition forms completed by ordering healthcare practitioners, with the majority of patients ascertained on suspicion of HBOC.

**Results:** Over 98% of patients were female and 72% reported a personal history of cancer. The most frequent female cancer was breast (63%), followed by ovarian (8%). Males comprised <2% of the patients and greater than 50% of them reported a personal history of breast cancer. Overall, 12.7% of patients tested positive for a laboratory classified pathogenic variant. Pathogenic variants were enriched in patients with a personal history of cancer (13.9%) versus no personal cancer history (9.5%); this is especially striking in cancers directly associated with HBOC. Pathogenic variants were detected in patients originating from Europe, Asia, Africa and North and South America.

**Conclusion:** BRCA clinical testing is valuable at the global level for identifying high-risk patients who would benefit from increased surveillance and medical intervention.

#### P12.023

#### Germline and somatic BRCA1/2 mutations in ovarian cancer patients unselected for family history

*G. Rodella<sup>1</sup>, R. Zuntini<sup>1,2</sup>, G. Barbero<sup>1</sup>, M. G. Tedesco<sup>4</sup>, S. Miccoli<sup>1,2</sup>, L. Godino<sup>1,2</sup>, A. M. Perrone<sup>2,3</sup>, D. Santini<sup>2,4</sup>, B. Corti<sup>6</sup>, C. N. Salfi<sup>4</sup>, F. Buscherini<sup>1</sup>, S. Ferrari<sup>1,2</sup>, P. De Iaco<sup>2,3</sup>, C. Zamagni<sup>2,5</sup>, D. Turchetti<sup>1,2</sup>,*

<sup>1</sup>Unit of Medical Genetics, Department of Medical and Surgical Sciences, Policlinico Sant'Orsola-Malpighi, Bologna, Italy, <sup>2</sup>Centro di Ricerca sui Tumori Ereditari, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italy, <sup>3</sup>SSD Oncologia Ginecologica, AOU di Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy, Bologna, Italy, <sup>4</sup>SSD UO Anatomia Patologica, AOU di Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy, Bologna, Italy, <sup>5</sup>Oncologia Medica, AOU di Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy, Bologna, Italy.

Ovarian Cancer (OC) is the seventh most common cause of cancer-related death in women. It is estimated that about 15% of OC arise in women carrying germline BRCA1/2 mutations and that 3% of sporadic serous OCs harbour somatic BRCA1/2 mutations; BRCA deficiency is predictive of response to PARP-inhibition.

Since July 2015, we have included for BRCA1/2 testing also women with high grade OC with no suggestive family history. Among 21 patients with apparently sporadic OC who have undertaken testing to date, complete results are available for 12; four of those (33%) carry a deleterious mutation (three in BRCA2, one in BRCA1). In addition, we undertook a research project aimed at analysing BRCA1/2 in fresh-frozen tissue of newly diagnosed OC patients. So far, 15 patients have been enrolled; BRCA1 sequence analy-

sis allowed to identify three mutations out of 14 tested tumors (21%), all showed LOH; moreover, one mutation was found in one out of 13 tumors sequenced for BRCA2, which failed to show LOH. All BRCA1/2 sequence mutations were subsequently found to be germline. BRCA1 MLPA analysis was performed in 11 tumors and detected 8 acquired deletions of the whole gene (three explaining LOH in mutation carriers). Out of eight tumors analyzed with BRCA2 MLPA, two showed deletion of the whole gene (one in a tumor also showing loss of BRCA1). Although preliminary, our results support a relevant frequency of germline BRCA1/2 mutations in OC patients, while somatic mutations appeared to be limited to monoallelic BRCA1 and/or BRCA2 loss.

#### P12.024

#### Prevalence of pathogenic gene variants associated with breast and ovarian cancer in Estonia

*H. Roomere, Ü. Murumets, P. Laidre, T. Kahre;*

*Tartu University Hospital, United Laboratories, Department of Genetics, Tartu, Estonia.*

**Introduction:** Breast cancer (BC) is the most common cancer among Estonian women, 650 new cases are diagnosed annually. Ovarian cancer (OC) is less frequent, 150 new cases per year. ~10% of cancers are associated with hereditary predisposition.

**Materials and Methods:** Altogether 953 individuals (906 females, 47 males) were sent for genetic testing by oncologist or medical geneticist during 2008 - 2015. BC was diagnosed in 339 patients (36%) and OC in 122 (13%), 14 patients had both cancers. 478/953 were high risk family members. 182 patients (19%) were 45y or younger. 654 (69%) had family history of BC/OC. BRCA1/2 genes were analysed either by APEX, Sanger or next generation sequencing (NGS). 314 patients were analysed against BC/OC high and medium risk genes by NGS. Deletions/duplications in BRCA1/2 genes were excluded by MLPA analysis in 547 patients.

**Results:** 158 patients (17%) were mutation-positive. The prevalence of BRCA1/BRCA2 mutations in BC/OC group (447 patients) was 18%, BRCA1 main mutations ratio was 45%. Interestingly we did not find any deletions/duplications from BRCA1/2 MLPA. In 26 cases (BRCA1/2 negative) the pathogenic variant was found either from PPMID, BRIP1, CHEK2, MSH2, MSH6, PMS1, CDH1, ATM or NBN gene.

**Conclusions:** Two main mutations in BRCA1 cover ~54% of all BRCA1/2 pathogenic mutations in Estonia: c.4035delA (21%); c.5266dupC (33%). Therefore we recommend start screening with detection of these two relatively common BRCA1 mutations and in case of negative result continue with NGS. BRCA1/2 MLPA analysis is not informative in Estonia.

#### P12.025

#### TruRisk® based next-generation sequencing and CNV detection demonstrate the importance of implementing other breast and ovarian cancer-associated genes in routine diagnostics

*L. Buelow, B. Bluemcke, E. Pohl, B. Versmold, A. Waha, J. Driesen, K. Keupp, A. Baasner, I. Praulich, J. Hauke, E. Hahn, B. Wappenschmidt, R. Schmutzler;*

*Center for Hereditary Breast and Ovarian Cancer, Cologne, Germany.*

Approximately 24% of familial breast cancer (BC) and/or ovarian cancer (OC) cases analyzed within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) are caused by pathogenic BRCA1/2 mutations. However, the mutation frequencies of non-BRCA1/2 genes associated with familial BC and/or BC/OC are largely unknown. Therefore, panel next-generation sequencing represents a comprehensive approach to simultaneously analyse numerous candidate genes regarding their involvement on BC/OC pathogenesis. Here, we present extended NGS data generated by using the GC-HBOC-designed TruRisk® gene panel. In this study, a cohort of 594 index cases from high-risk BC and BC/OC families, negative regarding deleterious mutations in BRCA1/2 was analyzed. Data analysis was accompanied by CNV detection using the SophiaDDM Version 3.5.0.12-p5.0.0 analysis tool (Sophia Genetics). By focusing on 13 BC and/or OC associated genes (ATM, CDH1, CHEK2, FANCM MLH1, MSH2, MSH6, NBN, PALB2, PTEN, RAD51C, RAD51D, TP53), we identified 25 different pathogenic variants, including large rearrangements, in 25 unrelated mutation carriers (4.2%). Mutations were identified in ATM (n=3), CHEK2 (n=10), FANCM (n=4), PALB2 (n=7) and RAD51C (n=1). 24% of these mutations are large rearrangements, found in ATM, CHEK2 and FANCM. Therefore, our study highlights the importance of comprehensive gene panel testing along with CNV detection to be included in BC and/or OC routine diagnostics. The results of the CNV detection together with further approximately 300 samples, which are already processed, will be evaluated.

**P12.027****Easy-to-use online self-test identifies women at high familial risk of breast cancer and decreases anxiety**

A. van Erkelen, N. Hoogerbrugge;

Radboud University Medical Center, Nijmegen, Netherlands.

**Introduction:** Individuals at an increased familial BC risk should be identified, as they and their relatives can receive earlier and more intensive BC surveillance. An online self-test for familial BC can identify women at increased familial risk and may decrease anxiety and distress even in patients at low risk.

**Methods:** Women invited for population-based screening mammography were also invited for an online self-test and questionnaires. Psychological impact was assessed at baseline (T0), immediately post-test (T1), after two weeks (T2) using the State-trait inventory Dutch Version (STAI-DY) and Hospital Anxiety and Depression Scale (HADS).

**Results:** In total, 304 completed T0 and T1, 195 completed T2. At T1, a moderate or high familial BC risk was identified in four (1%) and 18 (6%) women. There was a significant reduction in state anxiety at T1 and T2 (T1-T0: mean change = -1.69 (95%CI:-2.21,-1.17), P<0.001; T2-T0: mean change = -2.69 (95%CI:-3.93,-1.45), P<0.001). Similarly, trait anxiety but not distress were reduced at T2 (mean change = -1.31 (95%CI:-2.14,-0.48), P=0.002; mean change = -0.18 (95%CI:-0.78,0.43), P=0.57). Women at moderate or high familial BC risk had slightly higher levels of anxiety and distress at T0, T1 and T2, and similar decreases in psychological outcomes between T1-T0 and T2-T0.

**Conclusion:** An online self-test for familial BC identified women at moderate or high familial BC risk (prevalence 6%) and decreased anxiety. Adding familial risk assessment to population BC screening may prevent BC in relatives.

**P12.028****Founder BRCA1 and BRCA2 mutations in women with breast and/or ovarian cancer from Eastern Europe (Ukraine): application of next generation sequencing**A. Myszka<sup>1</sup>, H. Akopyan<sup>1,2</sup>, N. Kitsera<sup>2</sup>, F. Hammet<sup>3</sup>, H. Tsimiklis<sup>3</sup>, D. Park<sup>3</sup>, T. Nguyen-Dumont<sup>3</sup>, M. Southey<sup>3</sup>;

<sup>1</sup>Institute of Obstetrics and Emergency Medicine, University of Rzeszow, Rzeszow, Poland, <sup>2</sup>Institute of Hereditary Pathology of National Academy of Medical Sciences, Lviv, Ukraine, <sup>3</sup>The Genetic Epidemiology Laboratory, Department of Pathology, University of Melbourne, Melbourne, Australia.

Mutations in a small number of genes have been associated with a high risk of breast and ovarian cancer. The frequency of founder mutations in BRCA1 and BRCA2 genes can inform genetic testing strategies for some populations. The main purpose of the study was to search for new pathogenic mutations that are associated with high risk of breast and/or ovarian cancers in the Ukraine population.

The study group consisted of 132 women from West Ukraine diagnosed with breast cancer and/or ovarian cancer. All cases had a positive family history of breast and/or ovarian cancer. DNA samples were isolated from blood. Studies were performed using Hi-Plex technology incorporating massively parallel sequencing (next generation sequencing).

In the group of patients we detected 25 (19%) pathogenic mutations in BRCA1 (5 recurrent and 3 singular mutations) and 9 (7%) pathogenic mutations in BRCA2. The study identified a large number of variants in BRCA1 and BRCA2 that have uncertain or conflicting significance to breast and ovarian cancer risk.

This was the first study of women at high risk of breast and/or ovarian cancer from the West Ukraine population using next generation sequencing and established that there is a high frequency of BRCA1 and BRCA2 mutations in this population. The results will contribute to improved genetic testing in the Ukrainian population that currently relies on the testing of a limited selection of specific mutations in BRCA1 and BRCA2 in clinical genetics practice. Improved genetic risk assessment could also lead to improved cancer prevention in this population.

**P12.029****Germline BRCA1, BRCA2, NBN, CHEK2, TP53 mutations in the group of the young Polish patients diagnosed with breast cancer.**D. Nowakowska<sup>1</sup>, D. Czapczak<sup>2</sup>, U. Piekarska<sup>1</sup>, A. Kluska<sup>1</sup>, A. Janiec-Jankowska<sup>1</sup>;<sup>1</sup>The Maria Skłodowska-Curie Cancer Center & Institute in Warsaw, Warsaw, Poland,<sup>2</sup>The Maria Skłodowska-Curie Cancer Center & Institute of Oncology in Warsaw, Warsaw, Poland.

Breast cancer (BC) at young age is associated with poor prognosis, therefore there is a great need to characterize better this group of patients.

**Material/methods:** 334 women with BC at/under the age of 35 were of

fered genetic counseling and testing. The analysis was performed on DNA from the peripheral blood. The mutations in BRCA1,2 were detected using DHPLC and sequencing of exons in which the Polish founder mutations are found most frequently. TP53 gene was sequenced from exon 2 to 10. All the samples were screened for the Slavic c.657-661del5 mutation in NBN gene and for the most common mutations in CHEK2.

**Results:** In this group of 334 patients we identified 67(20%)BRCA1,2 mutation carriers and 3 TP53(0,9%), 3 NBN (0,9%), 3 CHEK2(0,9%) carriers. Majority of the BRCA1 mutations were founder Polish mutations. Most of the BRCA1 carriers had 1st and/or 2nd degree relatives with breast and/or ovarian cancer. There was no such correlation in the group of BRCA2 and TP53 mutation carriers, however all TP53 carriers had BC with high expression of estrogen and progesterone receptor and overexpression of Her2 („triple positive BC“). Conclusion: In Polish women diagnosed with BC at very young age genetic testing for the BRCA1,2 mutations should be offered, even in the absence of relevant family history. Screening for the founder Polish BRCA1 mutations is not sufficient in this group. In the youngest women diagnosed with „triple positive“ BC sequencing of TP53 gene should be considered. Genetic testing may guide important diagnostic and therapeutic decisions in this group of patients.

**P12.030****Identification of novel candidate genes for hereditary breast cancer via whole exome sequencing**D. Turchetti<sup>1,2</sup>, R. Zuntini<sup>1,2</sup>, F. Isidori<sup>1</sup>, C. Diquigiovanni<sup>3</sup>, F. Buscherini<sup>3</sup>, F. Palombo<sup>3</sup>, S. Miccoli<sup>1,2</sup>, E. Bonora<sup>1,2</sup>;

<sup>1</sup>Centro di Ricerca sui Tumori Ereditari, Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, BOLOGNA, Italy, <sup>2</sup>Genetica Medica, Policlinico S.Orsola-Malpighi, Bologna, Italy, <sup>3</sup>Genetica Medica, Policlinico S.Orsola-Malpighi, BOLOGNA, Italy.

Hereditary breast cancer (HBC) accounts for 5% of BC cases. Germline BRCA1/2 mutations explain 60% of HBC, but no other major genes have been identified in the remaining 40%.

We decided to perform whole exome sequencing (WES) in affected I-degree cousin-pairs displaying features suggestive of HBC, who tested negative for BRCA1/2. Based on their relationship, I-degree cousins share 1/8 of the entire genome, which helps filter and select candidate variants. To date, 4 cousin-pairs were analyzed through WES and candidate variants were identified in three different genes in 3 pairs, whereas the fourth one shared a BRCA2 deletion not previously identified by Sanger sequencing.

The candidate variants, affecting ROS1, RASAL1 and ICAM5 genes, were confirmed by direct sequencing. Further characterization of the variants is ongoing. In particular, the ROS1 variant affected a predicted canonical splice site, suggesting an effect on the correct splicing of ROS1, a tyrosine kinase receptor that has been implicated in several cancer types. We inserted the wild-type or mutant genomic region into a minigene plasmid, transfected it into COS7 cells, and found that the mutant cells used a cryptic intronic splice site 20 bp downstream, leading to the insertion of a premature stop-codon in the protein. Future studies will explore the effect of the variant on cell growth/proliferation and invasion in CRISP/Cre-modified MCF10A and MCF7 cells.

Our findings support previous evidence that many different genes account for the fraction of HBC not related to BRCA1/2 mutations and led us to identify promising novel variants for HBC.

**P12.031****Cluster analysis of breast cancer expression profiles and the search for genes associated with metastatic tumors**K. Grishina<sup>1</sup>, T. Muzaffarova<sup>1</sup>, N. Pospekhova<sup>1</sup>, S. Poyarkov<sup>1</sup>, E. Glubokova<sup>1</sup>, V. Khaylenko<sup>2</sup>, A. Karpukhin<sup>1</sup>;

<sup>1</sup>Research Centre for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>Blokhin Russian Cancer Research Centre, Moscow, Russian Federation.

The investigation of 75 gene quantitative expression levels in a sample of breast cancer tumors in relation to the normal breast tissue was performed. All patients of the sample were not subject to radiation, neither to chemotherapy. The set of 75 genes was formed on the basis of their participation in the progression of breast cancer. By cluster analysis 3 clusters of gene co-expression were identified. Two of them were enriched by metastasis cases. The frequency of metastasis cases was not significantly different in these clusters that allowed combining them (group 1). A statistically significant difference in the frequency of cases with metastasis in group 1 in relation to the cluster poor in metastasis (group 2) was shown (OR = 20, 95% CI = 1,9 to 218,4 p = 0,01). The 14 differentially expressed genes in group 1 relative to group 2 with significantly different expression level were identified. The 11 of them were differentially expressed in both clusters with metastasis and 3 were specifically expressed only in one cluster. The most highest le-

vel of gene expression revealed in one cluster MMP9 and FN1, and uPAR, PLAUR, ZEB2 in another cluster with metastasis. Excess of the expression level of these genes in cluster with metastasis relative to the group 2 is 10 - 50 times.

#### P12.033

#### Evaluation of circulating miRNAs expression as non-invasive biomarker for Breast Cancer in Iranian women

E. Soleimanpour<sup>1</sup>, M. Hosseinpour Feizi<sup>1</sup>, E. Babaei<sup>1</sup>, V. Montazeri<sup>2</sup>;

<sup>1</sup>Tabriz University, Tabriz, Iran, Islamic Republic of, <sup>2</sup>Noor Nejat Hospital, Tabriz, Iran, Islamic Republic of.

**Introduction:** Breast cancer is the most common cause of cancer mortality among women. So, early detection and targeted therapies of cancer can significantly reduce the mortality as well as increase the survival rate. MicroRNAs are a well-known candidate to develop predictive and prognostic biomolecules that can be affordably used as early and non-invasive detection marker. The aim of this study was to evaluate the expression levels of miR-21 and miR-155 in Iranian Breast cancer patients and the potential use of circulating miRs as non-invasive biomarker.

**Material and methods:** Tumor specimens, paired non-tumoral adjacent tissues and matching plasma samples from 30 Iranian women breast cancer were collected. Plasma of normal women were also used as control. The relative expression of miR-21 and miR-155 was measured by real-time quantitative reverse transcription PCR.

**Results:** The levels of miR-21 and miR-155 were significantly higher in plasma ( $p = 0.02$  and  $0.01$ , respectively) and tissue sample ( $p = 0.00$  and  $0.00$ , respectively) of breast cancer patients in comparison with control groups. Receiver operating characteristic curve analysis revealed 0.81 and 0.83 in miR-21 and miR-155 tissue, as well as 0.99 and 0.92 in miR-21 and miR-155 plasma samples respectively. Moreover, there was no relation between the expression level of miRs and clinic-pathologic data.

**Conclusion:** These finding revealed that the expression patterns of these circulating miRs in Iranian women are the same as their paired tissue groups and also other population over the world. So, circulating miR-21 and miR-155 can be used as non-invasive biomarker for breast cancer detection.

#### P12.035

#### Results of BRCA1 and BRCA2 mutation screening with next generation sequencing in a cohort of breast and ovarian cancer patients in Trakya region of Turkey

H. Tozkur<sup>1</sup>, S. Demir Ulusal<sup>1</sup>, E. Atlı<sup>1</sup>, A. Sezer<sup>2</sup>, H. Gürkan<sup>1</sup>, N. Tunçbilek<sup>3</sup>;

<sup>1</sup>Trakya University, Medical Faculty, Department of Medical Genetics, Edirne, Turkey,

<sup>2</sup>Trakya University, Medical Faculty, Department of General Surgery, Edirne, Turkey,

<sup>3</sup>Trakya University, Medical Faculty, Department of Radiology, Edirne, Turkey.

**Introduction:** The role of BRCA1 and BRCA2 gene mutations in breast and ovarian cancer risk is supported by a great number of studies worldwide. Here we report a novel mutation in BRCA2 gene in a patient with breast cancer and summarize the BRCA1/BRCA2 mutation screening results of 54 patients (2 males, 52 females) enrolled to our center between November 2014- October 2015.

**Methods:** Automated DNA isolation was performed from peripheral blood samples of 54 patients using EZ1 DNA isolation kit. Coding regions of BRCA1(NM\_007294.3) and BRCA2 (NM\_000059.3) genes were captured by Ion Ampliseq Library Kit V2.0. Target enrichment was performed by Ion One Touch OT2 200 kit followed by semiconductor sequencing on Ion Personal Genome Machine. Torrent Suite Software and Ion Reporter Software were used to analyze mutations. IGV was used to visualize the amplicons. At least 100X coverage of the bases in the targeted regions were accepted for precise analysis of the mutations. Sanger sequencing was performed to confirm the presence of the mutations and to screen the other family members if needed.

**Results:** In total, pathogenic mutations were found in 6 patients. NM\_007294.3(BRCA1):c.5266dupC(p.Gln1756Profs\*74) (2/54 patients), NM\_000059.3(BRCA2):c.67+1G>A (2/54 patients) and NM\_000059.3(BRCA2):c.1773\_1776delTTAT(p.Ile591Metfs) (1/54 patients) mutations were determined. A novel mutation, NM\_000059.3(BRCA2):c.721A>T(p.Lys241Ter) was found in a patient with breast cancer diagnosed at age 22.

**Conclusion:** Pathogenic mutation frequency was 11.11% in our cohort. We suggest that mutation screening of BRCA1 and BRCA2 genes by next generation sequencing is a practical method for genetic testing of high risk populations.

#### P12.036

#### Landscape of the mutation pattern in 4175 HBOC families using NGS panel

L. Castéra<sup>1,2,3</sup>, S. Krieger<sup>1,2,3</sup>, N. Goardon<sup>1</sup>, A. Ricou<sup>1</sup>, A. Rousselin<sup>1</sup>, A. Legros<sup>1</sup>, O. Bruet<sup>1</sup>, C. Quesnelle<sup>1</sup>, F. Domin<sup>1</sup>, C. San<sup>1</sup>, B. Brault<sup>1</sup>, R. Fouillet<sup>1</sup>, C. Abadie<sup>4</sup>, O. Béra<sup>5</sup>, P. Berthet<sup>1</sup>, O. Letac<sup>1</sup>, T. Frebourg<sup>6,7,3</sup>, D. Vaur<sup>1,2,3</sup>;

<sup>1</sup>Comprehensive Cancer Centre François Baclesse, Caen, France, <sup>2</sup>Normandy Centre for Genomics and Personalized Medicine, Caen, France, <sup>3</sup>Inserm U1079, Rouen, France,

<sup>4</sup>Comprehensive Cancer Centre Eugène Marquis, Rennes, France, <sup>5</sup>University Hospital, Fort de France, France, <sup>6</sup>Rouen University Hospital, Rouen, France, <sup>7</sup>Normandy Centre for Genomics and Personalized Medicine, Rouen, France.

In 2012, to optimize the molecular diagnosis of hereditary breast and ovarian cancer (HBOC), we validated a NGS-based routine screening based on the capture of 21 to 33 genes involved in HBOC and paired-end sequencing on Illumina platforms. The bioinformatic pipeline includes CASAVA, NextGENe, CNVseq and Alamut-HT and a custom interfaced database (CanDiD). Using this strategy, we have now analyzed 4175 patients and detected 722 (17 %) deleterious mutations. The majority of the mutations (83%) were found in BRCA1, BRCA2, CHEK2, PALB2, ATM, TP53, RAD51C, BRIP1, RINT1 with respective incidences of 4.6%, 4%, 1.2%, 1.1%, 1%, 0.6%, 0.5%, 0.4%, 0.3%. BRCA1 and BRCA2 exhibited 359 mutations including 26 large rearrangements. The 46 mutations detected in PALB2 were mostly found in women with a cancer over 40 years and the 21 RAD51C mutations preferentially in women with ovarian cancer. TP53 was mutated in 23/3865 index cases including only 4 characteristic Li Fraumeni families and preferentially in women with breast cancer before 31 years. These results demonstrate the efficiency of this NGS procedure to perform molecular diagnosis of HBOC, confirms the genetic heterogeneity of HBOC and show that the fraction of potentially deleterious mutations detected within the other genes than BRCA1 and BRCA2 justifies their analysis and additional studies to estimate the associated cancer risks.

#### P12.037

#### Parent of origin and prognosis in hereditary non-BRCA breast cancer in Sweden

C. Wendt<sup>1,2</sup>, A. Lindblom<sup>3,4</sup>, S. Margolin<sup>1,5</sup>;

<sup>1</sup>Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden,

<sup>2</sup>Department of Oncology, Karolinska Hospital, Solna, Stockholm, Sweden, <sup>3</sup>Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden,

<sup>4</sup>Department of Clinical Genetics, Karolinska University Hospital, Solna, Stockholm, Sweden, <sup>5</sup>Department of Oncology, Karolinska University Hospital, Solna, Stockholm, Sweden.

**Introduction:** Breast cancer prognosis is affected by tumour characteristics and adjuvant treatment. It has been suggested that prognosis in familial breast cancer could be influenced by parent of origin with a worse prognosis when breast cancer is inherited paternally. The aim of this study was to investigate parent of origin effects on prognosis in our cohort of non-BRCA hereditary breast cancer families in Stockholm, Sweden.

**Material and methods:** Pedigrees from 1782 families were eligible. Index patients were divided into two study groups, paternal and maternal inheritance. Tumour characteristics and survival data for the index patients were collected and analysed.

**Results:** In total 319 families fulfilled inclusion criteria, 229 and 90 index patients in maternal and paternal group respectively. Affected mothers were excluded to avoid bias. Median follow-up was 11 years. No significant difference in overall survival or recurrence-free survival between maternal and paternal inheritance of breast cancer was observed with hazard ratios 0.99 (95% CI: 0.54 to 1.80) and 1.22 (95% CI: 0.78 to 1.92) respectively.

**Conclusion:** We found no evidence for a worse prognosis with paternally inherited breast cancer compared to maternal inheritance. However, with this sample size only large differences in prognosis can be detected. In that perspective the tendency towards worse recurrence-free survival (HR 1.22, 95% CI: 0.78 to 1.92) in the group with paternal inheritance is interesting. Also notable is the skew distribution with a predominance of maternally inherited cases indicating that accuracy of self-reported family history is low for paternally inherited breast cancer.

#### P12.038

#### A potential role for germline RECQL mutations in breast and ovarian cancer susceptibility

K. De Leeneer<sup>1</sup>, G. Wieme<sup>1</sup>, L. Vergote<sup>1</sup>, I. Coene<sup>1</sup>, F. Malfait<sup>1</sup>, T. Van Maerken<sup>1</sup>, B. Poppe<sup>1</sup>, K. Claes<sup>2</sup>;

CMGG Ghent University Hospital, Ghent, Belgium.

Breast cancer is the most common malignancy among women and the contribution of hereditary susceptibility has been well recognized. However,

since the known moderate-to-high risk genes explain only 5% of breast cancer incidence, the identification of new genetic factors is crucial. Recently, RecQL helicase-like (RECQL) was identified twice as a breast cancer susceptibility gene by whole exome sequencing (Cybulski et al., 2015; Sun et al., 2015). No other data on the prevalence of RECQL mutations in breast cancer susceptibility are currently available.

We evaluated the complete coding and splice site regions of RECQL in 323 unrelated (BRCA1/2 and PALB2 negative) breast and ovarian cancer families referred to our center for genetic testing. Mutation detection was performed with a targeted resequencing approach starting with singleplex PCR, Nextera XT library preparation and sequencing by synthesis on a MiSeq instrument. Reads were mapped with the use of CLCbio v7 and called variants annotated by an automated pipeline using VEP and Alamut. Detected variants were confirmed with Sanger sequencing.

In total 12 unique sequence variants were identified, of these, two splice site variants (c.867+3A>T and c.1798-2 A>G) and one missense substitution predicted to be damaging (c.1114G>A; p.Val372Ile) in the helicase domain warrant further investigation. These 3 novel variants were detected in families with a strong predisposition for ovarian cancer, hinting towards a possible role for RECQL in ovarian cancer predisposition in the Belgian population. In a second stage of this study the prevalence of RECQL mutations will be evaluated in ovarian cancer families.

#### P12.039

#### Variants in eight cancer genes (AIP, ATM, BLM, BRIP, CHEK2, EXT2, FANCM and RECQL4) and breast cancer risk

M. Jakimovska<sup>1</sup>, K. Kubelka<sup>2</sup>, L. Stojanovska<sup>3</sup>, A. Arsovski<sup>3</sup>, M. Karagjozov<sup>2</sup>, D. Plaseska-Karanfilska<sup>1</sup>;

<sup>1</sup>Research Centre for Genetic Engineering and Biotechnology, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>Acibadem Sistina Hospital, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>3</sup>Re-Medika Hospital, Skopje, Macedonia, The Former Yugoslav Republic of.

Recently, a number of moderate and low susceptibility genes have been demonstrated to account for a significant breast cancer (BC) risk. In this study we have screened 12 variants in eight cancer genes (AIP, ATM, BLM, BRIP, CHEK2, EXT2, FANCM and RECQL4) among 180 familial breast cancer patients and 180 controls from the general population, using a multiplex single based extension method. We have previously detected these variants among BC patients during the panel-based NGS screening of 94 cancer genes. Based on ClinVar and/or type of the mutation, five variants were pathogenic, while seven were variants of unknown significance (VUS). All VUS were predicted as deleterious with at least two in silico prediction tools. The variants were either present with very low frequency (<0.3%) or were not found in ExAC database, with the exception of FANCM c.4799C>T (2.7% among Europeans). We have detected higher number of variants (n=27) among BC patients in comparison to the controls (n=13, p=0.0188). The most frequent variant was FANCM c.4799C>T (2.22% in BC and 1.11 in controls), followed by AIP c.47G>A, ATM c.7475T>G and AIP c.911G>A. FANCM c.1972C>T was found in one BC patient and two controls, BLM c.11T>C in one BC patient and one control, while BLM c.1642C>T, BRIP1 c.751C>T, CHEK2 c.1550G>A, EXT2 c.2034+1G>T and RECQL4 c.1868G>A were detected each in only one BC patient. Detection of higher number of variants in these eight genes among BC patients leads to the conclusion that these variants can be considered as risk factor for breast cancer.

#### P12.040

#### Impact of low penetrance variants on breast cancer morbidity

M. Ustinova, Z. Daneberga, M. Nakazawa-Miklaševiča, L. Kalniņa, E. Miklaševičs, J. Gardovskis;

Riga Stradiņš University, Institute of Oncology, Riga, Latvia.

Introduction: Four of low-penetrance variants were chosen to test their impact on breast cancer morbidity and prognosis in the given population.

Materials and Methods: Tested population consisted of 2,530 European descent breast cancer patients and 731 healthy gender matched controls. SNP genotyping was carried out by restriction fragment length polymorphism and TaqMan SNP genotyping assays. Data analysis was performed using the program Rv3.1.0.

Results: The diverse effect of variants on breast cancer risk was observed (Table1.).

Table 1. Case-control study results.

SNP	Zygosity	OR	95% CI	P-value
rs9693444	Heterozygous	1.39	1.11-1.75	0.005
	Homozygous	1.51	1.2-3.4	
rs1436904	Heterozygous	0.79	0.63-1	0.008

<b>rs616488</b>	Homozygous	0.62	0.45-0.87	
	Heterozygous	0.86	0.68-1.09	0.367
<b>rs204247</b>	Homozygous	0.84	0.57-1.25	
	Heterozygous	0.88	0.68-1.14	0.423
	Homozygous	1.04	0.75-1.45	

We observed notably protective effect in the case of rs1436904 positive and rs9693444 wild type genotype compared to rs1436904 wild type and rs9693444 positive allele combination (OR=0.54; 95% CI=0.39-0.73; p<0.001). Disease specific survival rates did not show significant prognostic impact for any of tested variants. Worse prognosis trend (p=0.0877) was observed for rs9693444 positive and rs1436904 wild type genotype compared to rs1436904 positive and rs9693444 wildtype genotype.

Conclusion: Rs9693444 proved association with increased breast cancer risk, rs9693444 has a protective effect, but rs616488 and rs204247 has no impact on breast cancer risk in given population.

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#### P12.041

#### Genomic analysis of dual breast cancer tumors for determination of tumor progression and evolution

G. Repiska<sup>1</sup>, T. Sedlackova<sup>2</sup>, M. Mego<sup>3</sup>, J. Radvanszky<sup>2</sup>, Z. Cierna<sup>4</sup>, M. Chovanec<sup>2</sup>, F. Duris<sup>5</sup>, N. Muratiova<sup>2</sup>, G. Minarik<sup>2</sup>;

<sup>1</sup>Comenius University in Bratislava, Faculty of Medicine, Institute of Physiology, Bratislava, Slovakia, <sup>2</sup>Comenius University in Bratislava, Faculty of Natural Sciences, Department of Molecular Biology, Bratislava, Slovakia, <sup>3</sup>Comenius University in Bratislava, Faculty of Medicine and National Cancer Institute, 2nd Department of Oncology, Bratislava, Slovakia, <sup>4</sup>Comenius University in Bratislava, Faculty of Medicine, Institute of Pathological Anatomy, Bratislava, Slovakia, <sup>5</sup>Comenius University in Bratislava, Faculty of Mathematics, Physics and Informatics, Department of Computer Science, Bratislava, Slovakia.

Introduction: Cancer as disease is characterized by extreme genetic heterogeneity. Also when it comes to specific cancer types the heterogeneity is still present not only on interindividual basis but also on intratumoral level. The genomic analysis of dual tumors originated from the same patient could bring new information in the field of detection of disease progression and tumor evolution.

Materials and Methods: Dual breast cancer tumors of 4 patients were analyzed with ultradeep genomic sequencing. For genomic sequencing TruSeq Amplicon Cancer Panel and MiSeq system were used. Gained genomic data were analyzed using BaseSpace standard analysis tool for TruSeq applications. After variant calling variants with frequency between 10% and 90% were compared between the two samples originated from single patient. Tumor specific variants were subsequently characterized in details.

Results: After variant calling from 147 to 172 variants were identified. From these 13 to 22 variants were present in 10 to 90% variant frequency and except by both tumor samples shared variants in all tumor pairs at least one single tumor specific variant associated with oncogenic effect was recorded. These were represented by variants in ERBB2 (p. V777L and p.L869R), TP53 (p.P152fs\*14 and p.Y205S) and PIK3CA (p.Q546E and p.H1047R) genes.

Conclusions: Identified shared as well as single tumor specific variants with confirmed oncogenic effect could be used in personalized disease progression molecular testing. Moreover, with addition of future prospectively gained biological samples and clinical information important knowledge about tumor evolution could be added.

#### Acknowledgements

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#### P12.042

#### Rescuing low tumor-content formalin-fixed paraffin embedded (FFPE) biopsies for whole exome sequencing by DEPArray™ digital sorting technology

C. Forcato<sup>1</sup>, J. Laliberte<sup>2</sup>, C. Bolognesi<sup>1</sup>, C. Schumacher<sup>2</sup>, G. Buson<sup>1</sup>, C. Mangano<sup>1</sup>, P. Tononi<sup>1</sup>, F. Fontana<sup>1</sup>, G. Medoro<sup>1</sup>, T. Harkins<sup>2</sup>, N. Manaresi<sup>1</sup>;

<sup>1</sup>Silicon Biosystems, Bologna, Italy, <sup>2</sup>Swift Biosciences, Ann Arbor, MI, United States.

Introduction: According to recent data from ongoing basket trials, about 1 in 5 patients presents with poor biopsies with small sample size, and/or low-tumor content due to admixture of normal cells, preventing them access to precision oncology. Here we describe a workflow for whole-exome sequencing (WES) of pure populations of tumor cells obtained from very low tumor-cellularity FFPE samples using DEPArray™ sorting technology.

Materials and Methods: A FFPE 50μm thick section from breast infiltrating ductal carcinoma, with 10% tumor cellularity, was dissociated into a cell suspension. Using DEPArray™ digital sorter, 419 cells from 100%-pure tumor and 497 cells from normal stromal subpopulations were recovered based on Keratin/Vimentin immunofluorescence and DNA content. After lysis, Illumina® compatible libraries were prepared from cell-sorted or DNA-extracted samples using Accel-NGS® 2S DNA Library Kit from Swift

Biosciences, amplified, enriched using SeqCap EZ MedExome enrichment kit (Roche) and sequenced on a HiSeq 2500.

Results: Matched stromal/tumor analysis of B-allele frequency of heterozygous SNPs precisely identified Loss-of-Heterozygosity (LOH) regions, as well as Copy-gain regions, both undetectable on genomic DNA extracted without sorting. We readily detected a clinically relevant homozygous (23/23=100% reads) TP53:p.L111R somatic mutation in a LOH region of sorted-tumor fraction, missed in unsorted gDNA, where mutation was not called as present in only 1 read out of 20 (5%).

Conclusions: DEPArray™ sorting combined with Accel-NGS® 2S library kit enables WES on pure tumor and stroma of FFPE samples, offering a clear picture of tumor-specific variants including LOH and copy-numbers regardless of tumor cellularity.

#### P12.043

#### Calreticulin gene mutation status in JAK2 and MPL mutation negative essential thrombocythemia and primary myelofibrosis patients

E. O. Ote<sup>1</sup>, Y. K. Terzi<sup>1</sup>, S. Karakus<sup>2</sup>, F. I. Sahin<sup>1</sup>;

<sup>1</sup>Baskent University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Baskent University Faculty of Medicine Department of Hematology, Ankara, Turkey.

Introduction: JAK2 and MPL mutations are being used for diagnosis of Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), but 30-40% of the patients do not carry any mutations of these genes. Recently, another gene called Calreticulin (CALR) has been reported to be involved in development of ET and PMF. In this study, we investigated the CALR mutation frequency, mutation types and their relevance to clinical findings.

Materials and methods: 16 ET and 4 PMF patients were enrolled in this study. All patients were negative for JAK2 V617F, MPL W515K/L and S505N mutations. We investigated CALR mutation status with Sanger sequencing method.

Results: The mutation rate was 25% in general. However, we did not find any mutations in patients with PMF. We detected three different mutations (31.25%) in 5 patients with ET. Three of the mutations were 52 base pairs (bp) deletion (Type I mutation, c.1092\_1143del), one was 5 bp insertion (Type II mutation, c.1154\_1155insTTGTC) and the last one was 46 bp deletion (c.1094\_1139del). All patients with Type I mutation were women and the mean age (23.3y) was found to be significantly lower compared to all groups ( $p=0.003$ ).

Conclusions: The results showed that the mutation rate and types were similar to the previous reports. New prospective studies with larger cohort will help us to understand the effect of CALR mutations in the development and prognosis of myeloproliferative neoplasms.

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#### P12.044

#### Germline alterations in cancer patients and unaffected individuals with family history

S. K. Karachanak-Yankova<sup>1</sup>, Z. Hammoudeh<sup>1</sup>, G. Antov<sup>1,2</sup>, S. Ivanov<sup>3</sup>, L. Balabanski<sup>3</sup>, R. Vazharova<sup>2</sup>, M. Malinov<sup>3</sup>, D. Toncheva<sup>1,3</sup>;

<sup>1</sup>Department of Medical Genetics, Medical Faculty, Medical University-Sofia, Sofia, Bulgaria, <sup>2</sup>Bulgarian Academy of Sciences, Institute of Genetics 'Academician Doncho Kostov', Sofia, Bulgaria, <sup>3</sup>Genomic laboratory, SBALGAR 'Malinov', Sofia, Bulgaria.

Introduction: Approximately 10% of all types of cancer are hereditary disorders due to germline mutations, which can be transmitted to the offspring. The aim of the present study is to detect germline cancer-associated alterations, which increase the cancer risk among carriers, using next-generation sequencing.

Materials and methods: We have analyzed 17 blood DNA samples from individuals with family history: 6 patients (4 -with breast cancer, 1 -with ovarian and 1 -with colorectal cancer) and 11 unaffected persons (10 with breast cancer relatives and 1 with colorectal cancer relatives). The analysis was performed using the TruSight Cancer Panel, which includes 94 genes and 284 SNPs associated with predisposition to cancer.

Results: We have detected the following alterations: (i) the colorectal cancer patient and the unaffected person with family history both carry probably pathological mutations in the APC gene, namely p.Glu1573LysfsTer3 and p.Ala1283Pro; (ii) the ovarian cancer patient carries the pathological p.Val1663LeufsTer6 mutation in the BRCA2 gene; (iii) three out of four breast cancer patients have pathological mutations in the following genes: TP53 (193H>LH), BRCA2 (p.Ala938ProfsTer21) and BRCA1 (p.Gly1232Glyfs). Among the 10 unaffected persons with breast cancer family history five

are carriers of pathological mutations in BRCA2: p.Thr3033AsnfsTer11 or p.Lys1590SerfsTer27.

Conclusion: In cases with cancer family history, the possibility to detect the pathological mutations running in the pedigree is very high among patients (5 out of 6) and healthy relatives (6 out of 11) and can be determined by using the TruSight Cancer Panel.

#### P12.045

#### A capillary electrophoresis-sequencing based screening solution for identifying and quantifying hotspot mutations in solid tumors

S. Jackson, A. Gerstner, K. Varma;

Thermo Fisher Scientific, South San Francisco, CA, United States.

Sanger sequencing by capillary electrophoresis (CE) offers many advantages over NGS screening, including simplified workflow, faster time-to-answer, less expense, and easier data analysis. Coupled with Applied Biosystems™ Minor Variant Finder (MVF) software to detect low abundance alleles, these features make front-line screening of solid tumors for oncogenic mutations by CE an attractive possibility. We have designed a panel for CE sequencing consisting of 26 amplicons covering 66 COSMIC somatic mutations that are most commonly found in tumor DNA samples. A total 1972 COSMIC sites are covered by the panel. We screened 44 solid tumor FFPE samples from various tissues for these alleles. We found 34 samples with known hotspot mutations. Allele frequencies determined by CE sequencing and MVF at these sites were highly correlative with Ion Torrent™ NGS-determined frequencies. Pathogenic alleles were detected down to frequencies of 5%. Furthermore, minor variants were detectable in as little as 100pg of input DNA. We demonstrated the utility of this approach by screening tissues with unknown mutation profiles with the panel and identified and quantified hotspot mutations in known tumorigenic genes. Therefore, CE sequencing followed by MVF analysis provides a fast and simple first-line screen for pathogenic alleles in FFPE tumor samples.

#### P12.046

#### Whole genome approaches reveal complex genomic rearrangements on a Mesothelioma tumor genome: preliminary findings of a cohort study

T. TUNCEL<sup>1</sup>, M. Metintas<sup>2</sup>, G. Ak<sup>2</sup>, & Dégirmenci<sup>1</sup>, H. Güneş<sup>1</sup>;

<sup>1</sup>Eskisehir Osmangazi University Medical Faculty, Department of Medical Biology, Eskisehir, Turkey, <sup>2</sup>Eskisehir Osmangazi University Medical Faculty, Department of Chest Diseases, Eskisehir, Turkey.

Malign Mesothelioma (MM) is an aggressive malignancy originating from pleura, pericardia, heart and abdomen layers. Asbestos exposure is the main risk factor for the MM. Individuals with asbestos exposure have the higher risk to get MM. Asbestos exposure has many known pathological effects to the cell, but its effect mechanism and impact on the human genome still remains unclear. It is known that cancer genomes possess many complex genomic anomalies and heterogeneity with patient specific patterns. As the genome-wide scanning technologies and their analyzing methods evolve, our understanding and knowledge of cancer genome structure gain a high-resolution perspective. In this study, we combined Whole genome SNP Array and Whole Genome Sequencing (WGS) methods to map a Mesothelioma genomes' complex genomic architecture. Pleural Tumor of a Malign Mesothelioma patient with known asbestos exposure used for genomic DNA isolation who diagnosed in Eskisehir Osmangazi University Medical Faculty Chest Diseases Department. Tumor genome scanned with a combined effort both SNP array and WGS, as a result, we discovered both massive intrachromosomal structural anomalies affecting several chromosomes within Chromothripsis patterns on chromosomes 10, 12, 14 and inter-chromosomal rearrangements resulting formation of fusion genes including WDR70-NXPH1, SKA3-DDX10, TSHZ2-SLC35A1. We also detected copy number losses of some major cancer-related genes which are confirmed with both two methods. According to our results, we suggest that some genomic anomalies especially fusion genes can be implied as precision medicine targets for future.

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## P12.048

### Identification of microRNAs differentially expressed in Human Larynx Cancer Stem-Like Cells.

*M. Ozen<sup>1,2</sup>, O. Karatas<sup>3</sup>, I. Suer<sup>4</sup>, B. Yuceturk<sup>5</sup>, M. Yilmaz<sup>6</sup>, B. Oz<sup>7</sup>, G. Guven<sup>8</sup>, H. Cansiz<sup>6</sup>, C. Creighton<sup>9</sup>, M. Ittmann<sup>10,11</sup>,*

<sup>1</sup>Biruni University Medical School Department of Medical Genetics, Istanbul, Turkey,

<sup>2</sup>Istanbul University Cerrahpasa Medical School, Department of Medical Genetics, Istanbul, Turkey, <sup>3</sup>Molecular Biology and Genetics Department, Erzurum Technical University, Erzurum, Turkey, <sup>4</sup>Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Turkey, <sup>5</sup>Advanced Genomics and Bioinformatics Research Center, The Scientific and Technological Research Council of Turkey (TUBITAK), Kocaeli, Turkey, <sup>6</sup>Department of Otorhinolaryngology, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey, <sup>7</sup>Department of Pathology, Istanbul University, Cerrahpasa School of Medicine, Istanbul, Turkey, <sup>8</sup>Department of Medical Genetics, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey, <sup>9</sup>Department of Medicine and Dan L. Duncan Cancer Center Division of Biostatistics, Baylor College of Medicine, Houston, TX, United States, <sup>10</sup>Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, United States, <sup>11</sup>Michael E. DeBakey VAMC, Houston, TX, United States.

**Background:** microRNAs are associated with regulation of distinct physiopathological processes including development of normal stem cells and carcinogenesis. In this study we aimed to investigate microRNA profile of Cancer stem-like cells isolated from freshly resected larynx cancer (LCa) tissue samples.

**Materials and Methods:** CD133-high stem-like cells were isolated from freshly resected LCa tumor specimens. MicroRNA profile of 12 pairs of CD133-high and CD133-low cells was determined using microRNA microarray and differential expressions of selected microRNAs were validated by quantitative real time PCR (qRT-PCR).

**Results:** MicroRNA profiling of CD133-high and CD133-low LCa samples revealed that miR-26b, miR-203, miR-200c, and miR-363-3p were significantly downregulated and miR-1825 was upregulated in CD133high larynx CSLCs. qRT-PCR analysis in a total of 25 CD133-high/ CD133-low paired samples were confirmed the altered expressions of these five microRNAs. Expressions of miR-26b, miR-200c, and miR-203 were significantly correlated with miR-363-3p, miR-203, and miR-363-3p expressions, respectively. Furthermore, in silico analysis revealed that these microRNAs target both cancer and stem-cell associated signaling pathways.

**Conclusions:** Our results showed that certain microRNAs in CD133-high cells could be used as cancer stem cell markers. Based on these results, we propose that this panel of microRNAs might carry crucial roles in LCa pathogenesis through regulating stem cell properties of tumor cells.

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## P12.049

### A long-term multidisciplinary surveillance program for patients with the Hereditary Diffuse Gastric Cancer Syndrome.

*P. R. Benusiglio, D. Lejri, M. Di Maria, P. Burtin, O. Caron, D. Malka;  
Gustave Roussy Cancer Campus, Villejuif, France.*

**Introduction.** The Hereditary Diffuse Gastric Cancer Syndrome (HDGC) is caused by germline mutations in the E-cadherin gene CDH1. Carriers have a high risk of signet-ring cell carcinoma (SRCC) and lobular breast cancer (LBC, women only). They require long-term multidisciplinary management, as stated in the latest international guidelines, and ideally in centres of excellence. We are about to set up a systematic surveillance program for carriers, and therefore needed a clear picture describing their management at our Institution. **Methods.** We collected data for patients who had tested positive for a CDH1 mutation at the Gustave-Roussy-Cancer-Campus-Grand-Paris and who were alive as of 11th-Feb-2016. Results. We identified twenty carriers from seven families. Five have a history of SRCC, one of LBC. Of the fifteen with no SRCC history, ten have already had risk-reducing gastrectomy (RRG), three are planning to do so, one is refusing the procedure and one has not collected his test results. Carriers with a history of cancer benefit from regular surveillance. The picture is however more complex for unaffected carriers. For example, follow-up ceased about six months after RRG for all but two. Regarding LBC, formal screening is rarely in place. Conclusion. There is room for improvement in the management of HDGC patients. We are setting up a long-term multidisciplinary surveillance program, in which we aim to address the following issues in particular: 1-the physical, metabolic, and quality-of-life consequences of RRG, 2-SRCC screening and incidence in patients delaying RRG, 3-LBC screening and incidence, and requests for risk-reducing mastectomy.

## P12.050

### Molecular basis of resistance of chondrosarcomas to conventional therapies

*J. J. Aury-Landas, N. Girard, M. Barreau, E. Lhuissier, K. Boumediene, C. Baugé;  
EA4652 - BioConnecT team - Université de Caen Normandie, Caen, France.*

Chondrosarcomas are malignant tumors of bone. They are considered as resistant to both chemotherapy and radiations. This emergent study aims to unravel the molecular mechanisms involved in the resistance of chondrosarcomas to cisplatin or to X-ray treatments by an innovative strategy of comparative functional genomics.

We observed, using five cell lines derived from human chondrosarcomas, that they had distinct responses to cisplatin or to X-ray treatments. To understand the molecular basis of these different sensitivities, we performed whole-exome sequencing on the cell lines. After strict filtration, 245 to 476 rare coding or splice variants per cell line were predicted to have a deleterious functional impact on the protein. We applied targeted, then pangenomic approaches to select relevant variants. We identified 66 mutated genes potentially implicated in the response to therapy. Interestingly, recurrent loss of function mutations of a tumor suppressor gene were identified in the three most resistant cell lines in which no apoptosis is induced by X-rays nor cisplatin. This gene is actionable by targeted chemotherapy. Functional analyses are in progress to validate the role of this very promising gene mutations and of the 65 other genes in resistance to treatments.

In conclusion, chondrosarcoma cell lines respond differently to conventional therapies. In addition, our study is the first one which extensively characterized commonly used human chondrosarcoma cell lines by whole-exome sequencing. Our preliminary results provide essential genetic information on resistance mechanisms through the identification of genes potentially involved in the response to cisplatin and X-ray radiations.

## P12.051

### TSC2 mutations in a chromophobe renal cell carcinoma patient with extraordinary response to temsirolimus.

*J. Roldán-Romero<sup>1</sup>, J. Maroto<sup>2</sup>, G. Anguera<sup>2</sup>, M. Apellániz-Ruiz<sup>1</sup>, F. Algaba<sup>3</sup>, C. Montero-Conde<sup>1,3</sup>, A. Cascón<sup>1,3,4</sup>, M. Robledo<sup>1,3,4</sup>, C. Rodríguez-Antón<sup>1,3,4</sup>,*

<sup>1</sup>Heredity Endocrine Cancer Group, Human Cancer Genetics Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, <sup>2</sup>Department of Oncology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>Department of Pathology, Fundació Puigvert, Barcelona, Spain, <sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

**Background:** Temsirolimus, an inhibitor of the mammalian target of rapamycin (mTOR), is an effective anti-cancer drug. There are large variations in response among patients, in part explained by mutations activating mTOR pathway. Here, we present a metastatic chromophobe renal cell carcinoma patient with complete response at metastatic sites and 80% size reduction of the renal tumor after temsirolimus treatment. After nephrectomy and 5 years after temsirolimus the patient developed a retroperitoneal adenopathy that was resected. Currently, >7 years after temsirolimus, the patient is disease-free.

**Patients and Methods:** Whole exome sequencing was performed on the primary tumor, the retroperitoneal adenopathy and normal blood. Somatic single nucleotide variants and small insertions or deletions leading to non-synonymous coding or splice site variants were identified. Immunohistochemical detection of TSC2 and phospho-ribosomal protein (pS6) was performed.

**Results:** Whole exome sequencing revealed two concurrent inactivating mutations in tuberous sclerosis 2 (TSC2), a critical negative regulator of mTOR Complex 1. One was a splicing defect (c.5069-1G>C) in intron 39 of TSC2, and the other consisted on two nucleotide substitutions in exon 28 (c. 3200\_3201delinsAA; p.V1067E). These mutations abolished TSC2 activity and increased mTOR pathway activation, as demonstrated by increased phosphorylation of S6, suggesting they constituted the basis for the exceptional temsirolimus response.

**Conclusions:** Whole exome sequencing in patients with extraordinary drug responses have the potential to uncover mechanisms that underlie drug sensitivity. Specifically, sequencing of mTOR pathway in chromophobe renal cell carcinoma may serve to prioritize mTOR inhibitor treatment and guide therapy in these patients.

## P12.052

### Prognostic impact of chromosomal translocations in myelodysplastic syndromes and chronic myelomonocytic leukemia patients. A study by the spanish group of myelodysplastic syndromes

**D. Costa<sup>1</sup>, X. Calvo<sup>1</sup>, A. Pereira<sup>1</sup>, A. Carrió<sup>1</sup>, F. Solé<sup>2</sup>, E. Luño<sup>3</sup>, J. Cervera<sup>4</sup>, T. Vallespi<sup>5</sup>, C. Muñoz<sup>1</sup>, C. Gómez<sup>1</sup>, A. Arias<sup>4</sup>, E. Such<sup>4</sup>, G. Sanz<sup>6</sup>, J. Grau<sup>6</sup>, A. Insunza<sup>7</sup>, M. Calasanz<sup>8</sup>, M. Ardañaz<sup>9</sup>, J. Hernández<sup>10</sup>, G. Azaceta<sup>11</sup>, S. Alvarez<sup>12</sup>, J. Sánchez<sup>13</sup>, M. Martín<sup>14</sup>, J. Bargay<sup>15</sup>, V. Gómez<sup>16</sup>, C. Cervero<sup>17</sup>, M. Allegue<sup>18</sup>, R. Collado<sup>19</sup>, E. Campo<sup>1</sup>, J. Esteve<sup>1</sup>, B. Nomdedeu<sup>1</sup>, M. Nomdedeu<sup>1</sup>;**

<sup>1</sup>Hospital Clinic, Barcelona, Spain, <sup>2</sup>Josep Carreras Leukemia Research, Badalona, Spain,

<sup>3</sup>Hospital Central de Asturias, Oviedo, Spain, <sup>4</sup>Hospital Universitario La Fe, Valencia, Spain, <sup>5</sup>Hospital Vall d'Hebrón, Barcelona, Spain, <sup>6</sup>Hospital Germans Trias i Pujol, Badalona, Spain, <sup>7</sup>Hospital Marqués de Valdecilla, Santander, Spain, <sup>8</sup>Universidad de Navarra, Pamplona, Spain, <sup>9</sup>Hospital Txagorritxu, Vitoria, Spain, <sup>10</sup>Hospital Universitario de Salamanca, Salamanca, Spain, <sup>11</sup>Hospital Clínico Universitario de Zaragoza, Zaragoza, Spain, <sup>12</sup>Centro Nacional de Investigaciones Oncológicas, Madrid, Spain, <sup>13</sup>Hospital Universitario Reina Sofía de Córdoba, Córdoba, Spain, <sup>14</sup>Hospital Doce de Octubre, Madrid, Spain, <sup>15</sup>Hospital Son Llátzer, Mallorca, Spain, <sup>16</sup>Hospital Universitario de la Princesa, Madrid, Spain, <sup>17</sup>Hospital Virgen de la Luz, Cuenca, Spain, <sup>18</sup>Complejo Hospitalario de Pontevedra, Pontevedra, Spain, <sup>19</sup>Hospital General de Valencia, Valencia, Spain.

**Introduction:** Chromosomal translocations are rare in the myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML). With the exception of t(3q), translocations are not explicitly considered in the cytogenetic classification of the IPSS-R and their impact on disease progression and patient survival is unknown. The present study was aimed at determining the prognostic impact of translocations in the context of the cytogenetic classification of the IPSS-R.

**Material and Methods:** We evaluated 1,653 patients from the Spanish Registry of MDS diagnosed with MDS or CMML and an abnormal karyotype by conventional cytogenetic analysis.

**Results:** Translocations were identified in 168 patients (T group). Compared with the 1,485 patients with abnormal karyotype without translocations (non-T group), the T group had a larger proportion of patients with refractory anemia with excess of blasts and higher scores in both the cytogenetic and global IPSS-R. Translocations were associated with a significantly shorter survival and higher incidence of transformation into AML at univariate analysis but both features disappeared after multivariate adjustment for the IPSS-R cytogenetic category. Patients with single or double translocations other than t(3q) had an outcome similar to those in the non-T group in the intermediate cytogenetic risk category of the IPSS-R.

**Conclusion:** The presence of translocations identifies a subgroup of MDS/CMML patients with a more aggressive clinical presentation that can be explained by a higher incidence of complex karyotypes. Single or double translocations other than t(3q) should be explicitly considered into the intermediate risk category of cytogenetic IPSS-R classification.

## P12.053

### Germline mutational landscape of Chronic Lymphocytic Leukemia

**A. Prasad<sup>1,2,3</sup>, G. Escaramis<sup>1,2,3</sup>, H. Susak<sup>1,2,3</sup>, M. Bossio<sup>1,2,3</sup>, L. Domenech<sup>1,2,3</sup>, O. Drechsler<sup>1,2,3</sup>, G. Castaño<sup>4</sup>, R. Rabionet<sup>1,2,3</sup>, I. Gut<sup>5</sup>, M. Kogevinas<sup>4</sup>, E. Campo<sup>6</sup>, C. Lopez-Otin<sup>7</sup>, S. Ossowski<sup>1,2,3</sup>, X. Estivill<sup>1,2,3</sup>;**

<sup>1</sup>Centre for Genomic Regulation, Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>CIBER in Epidemiology and Public Health (CIBERESP), Barcelona, Spain, <sup>4</sup>Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain, <sup>5</sup>National Centre of Genomic Analysis (CNAG), Barcelona, Spain, <sup>6</sup>Clinic Hospital (IDIBAPS), Barcelona, Spain, <sup>7</sup>University of Oviedo, Molecular Cancer Institute, Oviedo, Spain.

Chronic lymphocytic leukemia (CLL) is a cancer of B-lymphocytes, which expands in the bone marrow, lymph nodes, spleen and blood. Many genes have been implicated in CLL development through sequencing the tumor genome. However, these studies do not completely explain the pathogenesis of the disease, nor do they define the early events that lead the B-cells to accumulate the different types of somatic mutations leading to CLL. The aim of this project is to identify the landscape of germline risk factors that can predispose an individual to CLL. In particular, we have designed a rare variant association study based on the hypothesis that rare mutations are more likely to affect the function of the proteins and may modulate risk for complex diseases. We have analyzed exome-sequencing data on 450 CLL cases (normal and tumor samples) respectively, from the Spanish CLL ICGC consortium and performed rare variant association study with 950 controls from our in-house exomes. We identified genes that were enriched in our CLL cohort than in controls. We designed a targeted sequencing panel consisting of 358 genes, 945 cancer susceptible SNPs and 166 microRNAs. Sequencing was performed in a cohort of 95 CLL, 95 prostate cancer and 290 healthy control samples. The most relevant genes we have identified that are associated with susceptibility to CLL are *ERCC4*, *MET* and *NCAPG2*. The results imply an important possible component of CLL pathogenesis and

merit further functional studies for confirmation.

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## P12.054

### Detailed analysis of chronic lymphocytic leukemia cases with single TP53 mutation

**K. Plevova<sup>1,2</sup>, J. Malcikova<sup>1,2</sup>, J. Kotakova<sup>1,2</sup>, M. Trbusek<sup>2</sup>, J. Smardova<sup>2</sup>, E. Diviskova<sup>2</sup>, A. Oltova<sup>2</sup>, Y. Brychtaova<sup>2</sup>, M. Doubek<sup>1,2</sup>, S. Pospisilova<sup>1,2</sup>;**

<sup>1</sup>Central European Institute of Technology, Masaryk University, Brno, Czech Republic,

<sup>2</sup>University Hospital Brno, Brno, Czech Republic.

**Introduction:** In chronic lymphocytic leukemia (CLL), somatic defects of TP53 gene represent an adverse prognostic marker. In the majority of affected cases, TP53 is inactivated on both alleles due to the concurrent mutation and deletion. However, in ~30% of cases, only single TP53 mutation (TP53mut) without deletion is detected. We aimed to perform a detailed analysis of the second TP53 allele in these cases and to assess their genomic makeup.

**Materials and methods:** Separated CLL lymphocytes were investigated. TP53 mutations were analyzed using FASAY assay and direct sequencing. 17p13 deletions were assessed by iFISH. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix).

**Results:** We identified 179 CLL cases with TP53 disruption; 56/179 patients (31%) harbored single TP53mut with allelic frequency ranging 10-100%. We selected 22 cases with single TP53mut for CytoScan analysis. Interestingly, copy-neutral loss of heterozygosity (cn-LOH) in a subclone of CLL lymphocytes was detected in 11/22 cases. In 9/22 cases no abnormality in 17p locus was observed. In the remaining 2/22 cases, heterozygous deletion was newly detected. When we compared genomic complexity of leukemic clones with monoallelic vs biallelic (cn-LOH-only) TP53mut, the latter group exhibited significantly more genomic abnormalities (4 vs 6 chromosomal defects, p=0.0121). However, there was no difference in overall survival between the groups.

**Conclusion:** cn-LOH in 17p locus is a frequent finding in cases with single TP53mut leading to biallelic TP53 inactivation and should be considered in TP53 testing in CLL.

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## P12.055

### A heritable form of SMARCE1-related meningiomas with important implications for follow-up and family screening

**E. H. Gerkes<sup>1</sup>, J. Fock<sup>1</sup>, W. den Dunnen<sup>1</sup>, M. van Belzen<sup>2</sup>, C. van der Lans<sup>2</sup>, E. Hoving<sup>1</sup>, I. Fakkert<sup>1</sup>, M. Smith<sup>3</sup>, D. Evans<sup>3</sup>, M. Olderde-Berends<sup>1</sup>;**

<sup>1</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom.

**Introduction:** Recently, a new hereditary tumor predisposition syndrome has been discovered, resulting in an increased risk for spinal and intracranial clear cell meningiomas (CCMs) in young patients. Heterozygous loss-of-function germline mutations in the SMARCE1 gene are causative, giving rise to an autosomal dominant inheritance pattern.

**Case report:** We report on an extended family with a pediatric CCM patient and an adult CCM patient and several asymptomatic relatives carrying a germline SMARCE1 mutation.

**Discussion:** We discuss difficulties in genetic counseling for this heritable condition. Because of the few reported cases so far, the lifetime risk of developing meningiomas for SMARCE1 mutation carriers is unclear and the complete tumor spectrum is unknown. There is no surveillance guideline for asymptomatic carriers nor a long-term follow-up recommendation for SMARCE1-related CCM patients as yet. Until more information is available about the penetrance and tumor spectrum of the condition, we propose the following screening advice for asymptomatic SMARCE1 mutation carriers: neurological examination and MRI of the brain and spine, yearly from diagnosis until the age of 18 and once every 3 years thereafter, or in between if there are clinical symptoms. This advice can also be used for long-term patient follow-up. More data is needed to optimize this proposed screening advice.

## P12.056

### Spectrum of BCR-ABL Kinase domain mutations: A cohort study from Saudi Arabia

H. Abalkhail, S. Khalil;

King Faisal Special hospital & RC, Riyadh, Saudi Arabia.

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm (MPN) associated with a characteristic translocation between chromosome 9 and 22 to form Philadelphia chromosome (Ph). The introduction of tyrosine kinase inhibitors (TKIs) imatinib for CML marked a new era for targeted therapy. In general CML patients in chronic phase (CP) have a very good response to TKIs. A significant proportion harbor molecular residual disease and develop acquired resistance, in which mutations in the Abl kinase domain are the best known cause of resistance. In this retrospective study, we assessed the ABL kinase domain mutation in 165 patients referred from our clinics between 2011-2015 with Ph positive CML displaying either refractory to TKI or resistance displaying increase BCR-ABL1 levels monitored by Quantitative PCR. Mutation analysis was performed on RNA extracted from either blood or Bone marrows after amplification of the BCR/ABL1 transcript by nested PCR followed by Direct sequencing of the BCR-ABL1 Kinase domain including residues 243-487.

In total, 165 patients (78 Females and 87 males), Age between 10-80 years (median age: 38 years) of which 160 were adults and 5 pediatrics were analyzed. Among 1653 patients, 35 (21%) were positive for 14 different mutations across the ABL1 Kinase domain mutations (12 patients had T315I, 4 patients had the following: E255K and Y253H, 2 patients had each of the following mutation: H396R, F359 and F317L. Additionally, one patient with each of the following mutations: E355G, V299L, L248V, L298, Y326H, M244V, G250E and E453K).

## P12.058

### Exome sequencing data analysis to characterize rare germline copy number variants involved in colorectal cancer predisposition

S. Franch-Expósito<sup>1</sup>, C. Esteban-Jurado<sup>1</sup>, P. Garre<sup>2</sup>, I. Quintanilla<sup>1</sup>, S. Durán<sup>1</sup>, M. Cuatrecasas<sup>3</sup>, E. Samper<sup>4</sup>, J. Muñoz<sup>5</sup>, T. Ocaña<sup>1</sup>, S. Carballal<sup>6</sup>, M. López-Cerón<sup>1</sup>, A. Castells<sup>1</sup>, E. consortium<sup>1</sup>, M. Vila-Casadesús<sup>4</sup>, E. Serra<sup>5</sup>, S. Beltran<sup>5</sup>, J. Carvallo<sup>6</sup>, L. Bujanda<sup>7</sup>, C. Ruiz-Ponte<sup>8</sup>, J. Camps<sup>1</sup>, M. Gironella<sup>1</sup>, J. Lozano<sup>4</sup>, F. Balaguer<sup>1</sup>, J. Cubiella<sup>9</sup>, T. Caldés<sup>5</sup>, S. Castellví-Bel<sup>1</sup>;

<sup>1</sup>IDIBAPS, Hospital Clínic, CIBEREHD, Barcelona, Spain, <sup>2</sup>IdISSL, Hospital Clínico San Carlos, Madrid, Spain, <sup>3</sup>Pathology, Hospital Clínic, Barcelona, Spain, <sup>4</sup>Bioinformatics, CIBEREHD, Barcelona, Spain, <sup>5</sup>CNAG, Barcelona, Spain, <sup>6</sup>Centro Andaluz de Biología del Desarrollo, Sevilla, Spain, <sup>7</sup>Gastroenterology, CIBEREHD, Hospital Donostia, Donostia, Spain, <sup>8</sup>FPGMX, CIBERER, Santiago de Compostela, Spain, <sup>9</sup>Gastroenterology, CIBEREHD, Hospital de Ourense, Ourense, Spain.

**Introduction:** Colorectal cancer (CRC) represents the third most common cancer worldwide. On the other hand, copy number variants (CNV) are structural variations larger than 1 kilobase (kb) displaying copy number differences either polymorphic or related with predisposition to disease. **Materials and Methods:** Whole-exome sequencing (WES) was performed in germline DNA from 38 families with strong CRC aggregation without alterations in known hereditary genes. To identify rare CNV that could correspond to the mutational event for CRC predisposition, WES data was analyzed with ExomeDepth and CoNIFER tools. Variants shared by family members were prioritized and compared with the Database of Genomic Variants catalogue and our Spanish database. Variants were selected and validation and as well as segregation analysis were performed by comparative genomic hybridization (CGH). Gene expression analysis (arrays and qRT-PCR) were conducted in both germline and tumor cDNA and tumor immunohistochemistry (IHC) was also performed.

**Results:** Sixteen candidate CNV were detected in 15 families. The most interesting CNV corresponded to a duplication in chromosome 1 spanning 400 kb that was confirmed by CGH and family segregation was correct. TTF2, TRIM45, VTCN1 and miR942 were affected by the duplication. Gene expression data indicated TTF2 and miR942 overexpression in duplication carriers. Tumor IHC showed TTF2 protein overexpression and underexpression of the TMEM158 protein, a predicted target of miR942.

**Conclusions:** The duplication in chromosome 1 could correspond to the mutational event involved in the predisposition to CRC in the carrier family by overexpressing TTF2 and miR942, which would lead to TMEM158 under-expression.

## P12.059

### Aberrant DNA methylation in inherited and sporadic colorectal cancer

N. Sahnane<sup>1</sup>, F. Magnoli<sup>1</sup>, B. Bernasconi<sup>1</sup>, M. Tibiletti<sup>1</sup>, M. Pedroni<sup>2</sup>, M. Ponz de Leon<sup>2</sup>, G. Magnani<sup>2</sup>, C. Romualdi<sup>3</sup>, L. Reggiani-Bonetti<sup>2</sup>, L. Bertario<sup>4</sup>, S. Signoroni<sup>4</sup>, F. Sessa<sup>1</sup>, D. Furlan<sup>1</sup>, Italian Association for the Study of Familial and Hereditary Gastrointestinal Tumors (AIFEG);

<sup>1</sup>University of Insubria, Varese, Italy, <sup>2</sup>University of Modena-Reggio Emilia, Modena, Italy, <sup>3</sup>CRIBI Biotechnology Center, University of Padova, Padova, Italy, <sup>4</sup>National Cancer Institute-Milano, Milan, Italy.

**Introduction:** Aberrant DNA methylation has been widely investigated in sporadic colorectal carcinomas (CRCs) but less information is available about the role of epigenetics in hereditary CRC.

**Patients and Material:** LINE-1 hypomethylation and gene-specific hypermethylation of 38 promoters were analyzed in multicenter series of 220 CRCs including 71 microsatellite unstable Lynch CRCs (LS-MSI), 23 CRCs of young patients without inherited syndromes (EO-MSS), and 126 sporadic CRCs, comprising 28 MSI-unstable (S-MSI) and 98 microsatellite-stable cases (S-MSS). All tumor methylation patterns were integrated with clinico-pathological and genetic characteristics, namely chromosomal instability (CIN), TP53 loss, BRAF, and KRAS mutations.

**Results:** LS-MSI mainly displayed absence of extensive DNA hypo- and hypermethylation. A subset of LINE-1 hypomethylated LS-MSI showed G>A transition in the KRAS gene, absence of CIN and TP53 loss and an unfavorable prognosis. S-MSI exhibited extensive gene hypermethylation, MLH1 methylation, BRAF mutation and absence of CIN and TP53 loss. By contrast, S-MSS showed marked LINE-1 hypomethylation and CIN and they had a worse prognosis. EO-MSS were a genetically and epigenetically heterogeneous group. Like LS-MSI, some EO-MSS displayed low rates of DNA hypo- or hypermethylation and frequent KRAS G>A transitions. By contrast, some EO-MSS showed similar features to those observed in S-MSS, such as LINE-1 hypomethylation, CIN and TP53 deletion. In all four classes, hypermethylation of ESR1, GATA5, and WT1 was very common.

**Conclusion:** Aberrant DNA methylation analysis allows the identification of different subsets of CRCs. This study suggests that LINE-1 hypomethylation may be a useful prognostic marker in both sporadic and inherited CRCs.

## P12.060

### Prognostic assessment of colorectal cancer using clustering analysis

N. I. Pospekhova, Y. A. Shelygin, S. I. Achkasov, O. I. Sushkov, S. A. Frolov, O. A. Mainovskaya, A. A. Ponomarenko, A. S. Tsukanov, V. P. Shubin; State Scientific Centre of Coloproctology, Moscow, Russian Federation.

Colorectal cancer (CRC) is a clinically and molecularly heterogeneous disease. Different subtypes of tumors have different prognosis and sensitivity to target and chemotherapy. The aim of the work was to determine different prognostic subgroups keeping in mind cancer with peritoneal carcinomatosis (PC) to be the worst prognosis group.

**Methods.** We analyzed samples obtained from 41 patients with PC and 58 CRC stage I-III including 7 patients with Lynch syndrome (LS). 12-gene expression signature has been carried out for all tumors by RT-PCR. RAS/BRAF mutations and MSI were analyzed by sequencing and fragment analysis. Hierarchical clustering has been used for data analysis.

**Results.** Cluster analysis identified 4 groups of samples. Genes SFRP2, TNC, ZEB1, MUC2, VIM were the most significant for clustering. Seven cases have been included in Cluster 1 (MUC2/TFF3 presence, SFRP2/ZEB1 absence); 36 cases - Cluster 2 (TNC presence, MUC2/TFF3 absence); 24 - Cluster 3 (SFRP2/TNC/VIM presence, MUC2/TFF3 absence); 32 - Cluster 4 (SFRP2/MUC2/TFF3 absence). Distribution of CRC with PC among clusters was 9.8%; 29.3%; 34.1%; 26.8% cases; CRC stage I-III - 5.1%; 41.4%; 17.1%; 36.2% cases, respectively. RAS mutations were more common in Cluster 1 and 3 whereas MSI-H in Cluster 2 and 4. Four from 5 BRAF-V600E were found in Cluster 3.

**Conclusion.** The prevalence CRC with PC in Cluster 3 and its association with BRAF-mutation confirms aggressiveness, high metastatic potential and poor prognosis of this tumor group. Molecular classification of CRC can serve for disease prognosis assessment suggesting specific treatment for each case.

## P12.061

### The Fanconi anemia DNA damage repair pathway in the spotlight for germline predisposition to colorectal cancer

C. Esteban-Jurado<sup>1</sup>, S. Franch-Expósito<sup>1</sup>, J. Muñoz<sup>1</sup>, T. Ocaña<sup>1</sup>, S. Carballal<sup>6</sup>, M. López-Cerón<sup>1</sup>, M. Cuatrecasas<sup>2</sup>, M. Vila-Casadesús<sup>3</sup>, J. Lozano<sup>4</sup>, E. Serra<sup>5</sup>, S. Beltran<sup>5</sup>, E. consortium<sup>1</sup>, A. Brea-Fernández<sup>4</sup>, C. Ruiz-Ponte<sup>5</sup>, A. Castells<sup>1</sup>, L. Bujanda<sup>6</sup>, P. Garre<sup>7</sup>, T. Caldés<sup>5</sup>, J. Cubiella<sup>8</sup>, F. Balaguer<sup>1</sup>, S. Castellví-Bel<sup>1</sup>;

<sup>1</sup>IDIBAPS, CIBEREHD, Hospital Clínic, Barcelona, Spain, <sup>2</sup>Pathology, Hospital Clínic,

Barcelona, Spain, <sup>3</sup>Bioinformatics, CIBEREHD, Barcelona, Spain, <sup>4</sup>CNAG, Barcelona, Spain, <sup>5</sup>FPGMX, CIBERER, Santiago de Compostela, Spain, <sup>6</sup>Gastroenterology, CIBEREHD, Hospital Donostia, Donostia, Spain, <sup>7</sup>IdISSC, Hospital Clínico San Carlos, Madrid, Spain, <sup>8</sup>Gastroenterology, CIBEREHD, Hospital de Ourense, Ourense, Spain.

**Introduction:** Germline mutations in genes from the Fanconi anemia (FA) DNA damage repair pathway are known to cause FA and breast and ovarian cancer predisposition. Very recently, mutations in BRCA2, FAN1 and BLM genes have been postulated to cause familial colorectal cancer (CRC).

**Material and methods:** Seventy-four patients from 40 families with strong CRC aggregation without alterations in the known hereditary genes were selected from four Spanish hospitals and the EPICOLON consortium. Whole-exome sequencing was performed and variant filtering selected only very rare alterations, shared by individuals from the same family, with a putative loss of function and located in genes with a role compatible with cancer.

**Results:** We detected enrichment for variants in the FA DNA damage repair pathway genes in our cohort since six families carried heterozygous, rare (<0.01%), potentially pathogenic variants located in BRCA2/FANCD1, BRIP1/FANCJ, FANCC, FANCE and REV3L/POLZ. Three of the variants corresponded to frameshift alterations and three to missense variants predicted to be deleterious. Somatic studies also revealed loss of heterozygosity for four of the variants. Interestingly, extracolonic neoplasms including gastric, breast, uterine, and prostate were present in five families.

**Conclusions:** Although further studies are needed to confirm its contribution, the FA DNA damage repair pathway could play an important role in the inherited predisposition to CRC. It is important to highlight also that pleiotropy is becoming important in germline predisposition to cancer since a higher number genes may be involved in the genetic predisposition to a broader spectrum of neoplasms.

#### P12.062

##### Plasma RAS mutations for the selection and monitoring of colorectal cancer patients treated with anti-EGFR therapy

*A. Dalmases<sup>1</sup>, J. Vidal<sup>1</sup>, M. Vela<sup>1</sup>, M. Muset<sup>1</sup>, G. Piquer<sup>1</sup>, L. Visa<sup>1</sup>, R. Correa<sup>1</sup>, G. López<sup>1</sup>, V. Sikri<sup>2</sup>, J. Albanell<sup>1</sup>, F. Jones<sup>2</sup>, C. Montagut<sup>1</sup>, B. Bellosillo<sup>1</sup>;*

<sup>1</sup>Hospital del Mar, Barcelona, Spain, <sup>2</sup>Sysmex Inostics Inc, Mundelein, IL, United States.

**Introduction:** RAS testing in metastatic colorectal cancer (mCRC) patients in circulating tumor DNA (ctDNA) is being investigated as a new method for detection and monitoring of actionable mutations.

**Material and Methods:** 38 mCRC patients with known RAS status in tissue sample were analysed. Plasma mutations were determined with Sysmex Inostics BEAMing34 RAS mutation panel and compared to standard-of-care (SOC) RAS testing in tissue samples.

**Results:** Plasma and tissue results showed a concordance of 89.5% (table). In 2 patients in which a RAS mutation was identified in plasma but not in tissue, one was treated with anti-EGFR therapy and did not respond; in the other, the mutation was also detected in tissue by BEAMing re-examination. In one of two cases in which a RAS mutation was detected in tissue but not in plasma, BEAMing determination in tissue confirmed the same mutation detected by SOC.

Longitudinal analysis of RAS mutations in serial plasma samples from 5 patients that progressed to anti-EGFR, showed emergence of RAS mutations in 2 patients (40%).

**Conclusions:** The high overall concordance between tissue and plasma BE-AMing determination supports the blood-based testing to determinate the eligibility of CRC patients for anti-EGFR treatment. The detection of RAS mutations in plasma at progression to anti-EGFR suggests that BEAMing 34 RAS mutation panel may be used to monitor resistance.

Plasma RAS Result	Tissue RAS Result		
	Positive	Negative	Total
Positive	17	2	19
Negative	2	17	19
Total	19	19	38

#### P12.063

##### Expression analysis of PTEN in colorectal cancer

*M. Nemati<sup>1</sup>, E. Sakhinia<sup>2</sup>, N. Ajami<sup>3</sup>, S. Rezapour<sup>2</sup>;*

<sup>1</sup>Tabriz Genetic Analysis Center (TGAC), Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of, <sup>2</sup>Department of Medical Genetics, Tabriz University of Medical Sciences, Tabriz, Iran, Islamic Republic of, <sup>3</sup>Department of Biology, Faculty of Basic Science, Khoj Payam Noor University, Khoj, Iran, Islamic Republic of.

**Introduction:** The tumor suppressor phosphatase and tensin homolog (PTEN) is involved in many important biological processes related to cell survival, proliferation, and growth. Reduced expression of the PTEN is observed in several human cancers, indicating a critical role in carcinogenesis and a potential as a tumor marker in cancer. In this prospective study, we compared PTEN expression levels in colorectal tumor tissues with adjacent

non-tumor tissues to assess the diagnostic value of this biomarker in colorectal cancer and its relationship with the clinicopathological features of patients.

**Methods:** Total RNA was extracted from 48 pairs of colorectal tumor tissue and adjacent non-tumor tissue. Afterwards cDNAs were synthesized and the expression level of PTEN was quantified by real time PCR. The Correlation between the expression level of PTEN and clinicopathological features was studied and the capability of PTEN to function as a CRC tumor marker was also explored.

**Results:** The expression levels of PTEN ( $P<0.001$ ) were significantly decreased in colorectal tumors compared to adjacent non-tumor tissues. The Receiver operating characteristic (ROC) curve analysis on PTEN showed that the area under the ROC curve was high (0.83). No correlation was observed between PTEN expression levels and clinicopathological features of patients.

**Conclusion:** Our results suggest that PTEN can serve as an important prognostic indicator in colorectal cancer and may provide valuable information to diagnosis and targeted therapy purposes.

#### P12.064

##### Expression of inhibitors of TRAIL apoptotic pathway in colorectal tumours

*S. Tsikalakis<sup>1</sup>, I. Chatziandreou<sup>1</sup>, O. Dimopoulou<sup>1</sup>, N. V. Michalopoulos<sup>2</sup>, G. Theodoropoulos<sup>2</sup>, E. Patsouris<sup>1</sup>, A. Saetta<sup>1</sup>;*

<sup>1</sup>National and Kapodistrian University of Athens, Medical School, Department of Pathology, Athens, Greece, <sup>2</sup>National and Kapodistrian University of Athens, Medical School, 1st Department of Propaedeutic Surgery Hippokrateion Hospital, Athens, Greece.

**Introduction:** TRAIL (TNF related apoptosis inducing factor) is a member of the tumor necrosis factor superfamily which has been considered as a promising drug target for cancer therapy due to its ability to selectively induce apoptotic death in transformed cells. Inhibitors of TRAIL induced apoptosis include c-FLIP, XIAP, Mcl-1, Bcl-2, Bcl-xL genes.

**Methods:** 106 colorectal tumours (63 males, 43 females, median age 70 years) along with 20 normal tissues were analysed using RT-PCR, for relative mRNA expression analysis with  $\Delta\Delta Ct$  method for total c-FLIP, XIAP, Mcl-1, Bcl-2, Bcl-xL. Statistical analysis was performed in order to correlate relative mRNA levels with clinicopathological characteristics and with previous data on relative mRNA expression of TRAIL, DR4, DR5, DcR1, and DcR2 genes.

**Results:** Elevated mRNA levels were observed as follows: Bcl-2 16%, Mcl-1 8.7%, XIAP 4.9% and total c-FLIP 2.2%. Additionally, reduced mRNA levels were observed for: total c-FLIP 88%, Bcl-2 84%, Bcl-xL 80%, XIAP 38.7% and Mcl-1 96%. XIAP reduced mRNA levels were correlated with older patients (over 70 years old) and with smaller tumour size, T1/2. Finally, TRAIL ligand mRNA levels were correlated with stage and DcR1 elevated mRNA levels with patients' age.

**Conclusions:** Apart from decoy receptors' over expression, a small subset of cases showed elevated mRNA levels of apoptosis resistance genes. Our results depict a potential significant role of XIAP expression in limiting tumour growth during colon tumorigenesis. TRAIL expression emerged as an early event, possibly delaying disease progression.

#### P12.065

##### Field synopsis of genetic variation and colorectal cancer: unified 2016 update

*J. Little<sup>1</sup>, E. Theodoratou<sup>2</sup>, W. Zheng<sup>3</sup>, Z. Montazeri<sup>1</sup>;*

<sup>1</sup>University of Ottawa, Ottawa, ON, Canada, <sup>2</sup>University of Edinburgh, Edinburgh, United Kingdom, <sup>3</sup>Vanderbilt University School of Medicine, Nashville, TN, United States.

**Background:** According to current predictions of global demographic changes, the WHO estimates a 77% increase in colorectal cancer (CRC) incidence and 80% increase in CRC-related deaths by 2030 (Bray et al., 2012). Identifying genetic variants that influence susceptibility to disease potentially can inform the development of approaches for primary and secondary prevention. Two groups have published field synopses on genetic variants associated with CRC, in JNCI in 2012 and Gut in 2013. These groups are now working together on an update.

**Methods:** We have searched all published (and some unpublished) genetic association data -including candidate gene and GWAS- for CRC to the end of 2015. Furthermore, we have access to 4 GWAS datasets. We are conducting meta-analyses. We have reached a consensus on the operationalization of the Venice guidelines on the credibility of genetic association, which had differed between the two previous field synopses.

**Results:** More than 200 genes in about 770 SNPs were identified as putatively associated with CRC. For 450 SNPs, there was a single study only; for 90 SNPs, two studies; and for 230 SNPs, 3 or more studies. Meta-analysis will be carried out for SNPs with 3 or more studies, and credibility of association assessed according to the Venice criteria.

Conclusions: The identification of genetic variants with influence on CRC risk may reflect an importance of genes involved in colorectal cancer risk. Our data should help results of genetic associations studies to be placed in context and interpreted appropriately and should help direct future research effort.

#### P12.066

#### Genomic imbalances in tumor and surrounding tissue detected by MLPA in Brazilian patients with colorectal cancer

M. Rocha<sup>1,2</sup>, A. T. Dias<sup>3</sup>, E. A. Zanardo<sup>2</sup>, T. V. M. M. Costa<sup>2</sup>, F. A. R. Madia<sup>2</sup>, J. Damasceno<sup>2</sup>, C. Milan<sup>2</sup>, F. Balsamo<sup>1</sup>, S. D. F. Boratto<sup>1</sup>, S. H. C. Horta<sup>1</sup>, C. E. R. Corsi<sup>1</sup>, B. A. Bianco<sup>1</sup>, D. M. Christofolini<sup>1</sup>, C. P. Barbosa<sup>1</sup>, J. Waisberg<sup>1</sup>, L. D. Kulikowski<sup>1,2</sup>;

<sup>1</sup>Faculdade de Medicina do ABC, São Paulo, Brazil, <sup>2</sup>Faculdade de Medicina da USP, São Paulo, Brazil.

Gains and losses of chromosome arms are usually detected in colorectal cancer (CRC) samples and may be linked to progression of this disease. Thus, we investigated specific genomic unbalances associated with CRC in Brazilian patients who underwent surgery without previous neoadjuvant treatment. The tumors were histologically classified as low and high grade according to Brazilian Society of Pathology. The total of 30 tissue samples from neoplastic and non-neoplastic colorectal tissues was obtained from 10 patients. Paired peripheral blood samples were used as controls. The DNA was extracted using the QIAamp DNA Blood Midi Kit (QIAGEN, Valencia, California) and investigated by MLPA method (MRC-Holland®, Amsterdam, The Netherlands) with combination of specific kits for CRC basic research (P413 and P146). The results were analyzed using the software GeneMarker® (SoftGenetics, LLC, State College, PA - [www.softgenetics.com](http://www.softgenetics.com)). The results revealed alterations of several different regions: genomic gains in the long arm of 20 chromosome including probes located in ADMR1, OPRL1, MAPRE1, TPX2, ZNF217 genes and gains in the short arm of 17 chromosome including MNT and OVCA2 gene. In addition we found losses in short arm of chromosome 8 including markers of CDCA2, NDX3, LPL genes and losses in long arm of 13 chromosome including RB1, DACH, PSPC, ZMYM2 genes. Unexpectedly, we also detected similar genomic imbalances in adjacent control tissues for some patients suggesting that there is a field of chromosomal instability surrounding the tumor. Therefore the accurate genomic characterization of these tissues is critical to screening the progression of this disease.

#### P12.067

#### Analysis of complex karyotypes in patients with haematological neoplasms

H. Podgornik, U. Mlakar, M. Sever, P. Černelč;

Department of Hematology, Ljubljana, Slovenia.

Background. Most studies define complex karyotype (CK) as  $\geq 3$  or  $\geq 5$  independent abnormalities. In the last few years a new definitions have arisen as structurally CK and monosomal (MCK). The mentioned CKs have been claimed to be the strongest predictor of an inferior outcome in patients with haematological neoplasms. We analysed subgroups of CKs in our patients. Methods. GTG banding, FISH and spectral karyotyping were combined to resolve karyotypes. CKs were subdivided according to the number of aberrations (3, 4,  $\geq 5$ ), to monosomal (M), and structurally rearranged (S).

Results. 147 CKs were observed in the last 4 years. The majority of cases (77%) corresponded to AML and MDS followed by CLL patients. Frequency of different subtypes of CKs is given in Table 1. 73% of CKs had  $\geq 5$  aberrations. 89% of them were also structurally rearranged. 56% of CKs corresponded to monosomal and structurally complex at the same time (CKMS). All monosomal CKs with  $\geq 5$  aberrations were also structurally rearranged (CKMS5). This was the main group of CKs in MDS and AML. Structurally rearranged karyotypes (CKS4 and CKS5) were significantly more frequent in CLL than in AML or MDS ( $P < 0.05$ ).

Conclusions. In MDS a distinction between CK with 3 and 4 aberrations is already crucial for prognostification. To give comprehensive information to clinicians CKs should be analysed even more extensively.

Frequency of CK subtypes.				
Karyotype	All patients (N=147)	AML (N=56)	MDS (N=46)	KLL (N=17)
CK3	1 (1%)	1 (2%)	0 (0%)	0 (0%)
CKS3	18 (12%)	8 (14%)	5 (11%)	2 (12%)
CKM3	4 (3%)	2 (4%)	2 (4%)	0 (0%)
CKMS3	2 (1%)	1 (2%)	1 (2%)	0 (0%)
CK4	2 (1%)	1 (2%)	1 (2%)	0 (0%)
CKS4	9 (6%)	2 (4%)	1 (2%)	4 (24%)
CKM4	2 (1%)	0 (0%)	1 (2%)	1 (6%)
CKMS4	2 (1%)	0 (0%)	2 (4%)	0 (0%)
CK5	2 (1%)	3 (5%)	3 (7%)	0 (0%)
CKS5	26 (18%)	3 (5%)	5 (11%)	4 (24%)
CKM5	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CKMS5	79 (54%)	35 (63%)	25 (54%)	6 (35%)

#### P12.068

#### Connexin43 Gap Junctions and Melatonin Receptors as Independent Marker for Prostate Cancer

A. Tavartkiladze<sup>1,2</sup>, R. Khutishvili<sup>1</sup>, D. Khijakadze<sup>2</sup>;

<sup>1</sup>Institute For Personalized Medicine, Tbilisi, Georgia, <sup>2</sup>Tbilisi State Medical University, Tbilisi, Georgia.

Gap junctions are intercellular channels that are formed from members of a family of proteins, the connexins (Cx). Gap junctions play an important role in vital functions, including the regulation of cell growth and cell differentiation. Here, we examined the expression of Cx43, a major Cx in prostate tissue, in 27 surgical specimens obtained from prostate cancer patients who underwent a primary surgical castration prior to hormonotherapy or radiotherapy treatments. The expression of Cx43 gap junctions was compared to the levels of testosterone, melatonin (MLT1), and erbB2 tyrosine kinase receptors. In addition, a panel of prostate cancer cell lines and a series of normal rat prostate tissues and rat prostate tumors induced in vivo by N-Methylnitrosurea were studied. We demonstrated that the lack of Cx43 gap junctions is a common feature of human prostate cancer tissues compared to nonneoplastic prostate tissues surrounding primary tumors. Cx43 gap junctions were not observed in prostate adenocarcinomas, and they seem to be independent of testosterone, melatonin, and erbB2 receptor status. In prostate cancer cell lines and rodent prostate carcinoma tissues, down-regulation of Cx43 occurs at the mRNA level, suggesting a transcriptional mechanism for the decrease of Cx43 protein in prostate cancer. In summary, this study provides evidence of decreased expression of Cx43 gap junctions in prostate cancer at various stages of progression as well as prostate cancer cell lines and raises the possibility that Cx43 may be a useful marker for detecting early oncogenesis in the prostate.

#### P12.069

#### Identification of two novel cases of constitutional mismatch repair deficiency syndrome in a spanish consanguineous family

Y. Rodriguez<sup>1</sup>, D. Parraga<sup>2</sup>, I. Rey<sup>3</sup>, M. Gallego<sup>3</sup>, N. Carrizo<sup>3</sup>, J. Perea<sup>4</sup>, O. Toldos<sup>4</sup>, M. A. Martinez Gonzalez<sup>1</sup>, J. L. Vivanco<sup>5</sup>, V. Perez Alonso<sup>5</sup>, L. Robles<sup>6</sup>, D. Rueda<sup>3</sup>;

<sup>1</sup>Pathology Department, Doce de Octubre Hospital, Madrid, Spain, <sup>2</sup>Heredity Cancer Genetic Diagnostics Laboratory, Madrid, Spain, <sup>3</sup>Heredity Cancer Genetic Diagnostics Laboratory, Doce de Octubre Hospital, Madrid, Spain, <sup>4</sup>Surgery Department, Doce de Octubre Hospital, Madrid, Spain, <sup>5</sup>Pediatric Oncology and Hematology Department, Doce de Octubre Hospital, Madrid, Spain, <sup>6</sup>Familial Cancer Unit, Doce de Octubre Hospital, Madrid, Spain.

Introduction: The Constitutional MisMatch Repair Deficiency (CMMRD) syndrome (OMIM #276300) is a rare autosomal recessive cancer predisposition disease caused by inactivating biallelic germline mutations in mismatch repair (MMR) genes. Carriers develop in their childhood hematologic malignancies, central nervous system tumors or colorectal tumors, as well as other neoplasia. Many patients overlap neurofibromatosis type I features such as cafe-au-lait spots.

Patient and Methods: MMR genes were studied by amplicon based NGS on a suspected CMMRD patient belonging to a consanguineous Spanish family. Directed genetic study was extended to other family members, collecting clinical and pathological data. Microsatellite Instability (MSI) status and immunohistochemical (IHC) studies on mismatch repair complex proteins was performed on tumor and normal tissues.

Results: Two siblings carrying homozygous nonsense mutation on MSH6 gene were identified: one developed an abdominal B-cell non-Hodgkin lymphoma and two lymphoblastic T-cell lymphoma, and the other a nephroblastoma. Both of them were diagnosed at early age and presented cafe-au-lait macules. IHC on different tumors showed absence of staining for the MSH6 protein both in malignant and normal tissues. However, MSI was only observed in tumor but not in blood or oral epithelium controls.

Conclusions: Our results confirm previous clinical and molecular observations for CMMRD patients. Remarkably both homozygous carriers scored positive for the indication criteria for CMMRD testing suggested by the European Consortium „Care for CMMRD“. Additional molecular studies are currently being performed in order to better characterized this poorly known cancer syndrome.

#### P12.070

#### Expression of Contactin 4 is associated with malignant behaviour in pheochromocytomas and paragangliomas

L. Evenepoel<sup>1,2</sup>, F. H. van Nederveen<sup>3</sup>, L. Oudijk<sup>4</sup>, T. G. Papathomas<sup>5,4</sup>, D. F. Restuccia<sup>4</sup>, E. J. T. Belt<sup>4</sup>, G. J. H. Franssen<sup>4</sup>, R. A. Feeders<sup>4</sup>, A. Perren<sup>6</sup>, S. van Eeden<sup>7</sup>, H. J. L. M. Timmers<sup>8</sup>, W. de Herder<sup>4</sup>, L. Vroonen<sup>9</sup>, S. Aydin<sup>10</sup>, M. Vikkula<sup>4</sup>, R. R. de Krijger<sup>4,11</sup>, W. N. M. Dinjens<sup>4</sup>, A. Persu<sup>2</sup>, E. Korpershoek<sup>4</sup>;

<sup>1</sup>de Duve Institute, Brussels, Belgium, <sup>2</sup>Institut de Recherche Expérimentale et Clinique, Brussels, Belgium, <sup>3</sup>Laboratory for Pathology, Dordrecht, Netherlands, <sup>4</sup>Erasmus MC Cancer Institute, Rotterdam, Netherlands, <sup>5</sup>King's College Hospital, London, United

Kingdom, <sup>6</sup>Clinical Pathology Division, Bern, Switzerland, <sup>7</sup>Academic Medical Center, Amsterdam, Netherlands, <sup>8</sup>Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, <sup>9</sup>Centre Hospitalier Universitaire de Liège, Liège, Belgium, <sup>10</sup>Cliniques universitaires Saint Luc, Brussels, Belgium, <sup>11</sup>Reinier de Graaf Hospital, Delft, Netherlands.

**Introduction:** Pheochromocytomas and paragangliomas (PPGL) are rare and mostly benign tumours. Approximately 10% of PPGL are malignant, as defined by the presence of metastases, i.e. chromaffin tissue at a location that usually does not contain chromaffin cells. However, up to 35% of tumours in patients carrying an SDHB mutation appears to be malignant. No reliable marker allows prediction of whether a PPGL is, or will become malignant. In addition, there are no curative treatments if metastases occur.

**Materials and methods:** In order to identify genetic markers allowing to distinguish benign from malignant tumours, 40 benign and 11 malignant PPGL were investigated for differences in mRNA expression with Affymetrix arrays. Expression data were normalized according to Affymetrix recommendations. Then, using Pomeo II (<http://pomeo2.bioinfo.cnio.es/>), a Limma t-test was performed, to assess which genes were differentially expressed between benign and malignant PPGL. Clustered and non-clustered analysis were performed, and 15 genes with a False Discovery Rate (FDR) below 0.05 and a relative overexpression ratio of at least 4 were found. They were further investigated using qRT-PCR, and immunohistochemistry on Tissue Micro Array including 88 benign and 13 malignant PPGL. Subsequently, slides of malignant and benign tumours (n=32 respectively) were stained.

**Results:** Contactin 4 (CNTN4) was significantly overexpressed (FDR=0.001, and p=0.004 for the IHC) in malignant compared to benign tumours.

**Conclusion:** CNTN4 overexpression is associated with malignant PPGL, and may thus predict metastatic dissemination in patients with PPGL. Notably, CNTN1 has been shown to be associated with malignancy in different tumours including gliomas.

#### P12.071

#### Panel gene testing identified PTEN mutation in family that did not fulfill criteria for clinical diagnosis. Are we considering PTEN screening as often as we should?

**D. Georgiou, V. Kiesel;**

*GeneHealth UK, Cambridge, United Kingdom.*

The majority of colorectal cancer is sporadic; however up to 10% may be inherited; with Lynch syndrome, Familial Adenomatous polyposis and MUTYH associated polyposis the most common known hereditary colorectal cancer syndromes. Other conditions, such as Cowden syndrome are rare causes of colorectal cancer and colorectal polyps; hence genetic testing is not routinely offered. Historically, genetic counselling has involved performing a risk assessment based on an individual's family history of cancer and sequential single gene testing. With the advent of panel testing it is becoming possible to simultaneously examine multiple genes, which is eliciting unusual phenotypes.

This case report presents an individual tested through GeneHealth UK due to a personal history of colorectal polyps and a family history of colorectal cancer and polyps. No history of learning difficulties, skin lesions, other cancers/tumours was noted. The proband was counselled and offered Bowel-Gene panel testing, which tests 11 genes associated with colorectal cancer and colorectal polyps using NextGeneration sequencing on an illumine platform. A pathogenic mutation was found in the PTEN gene, which confirmed a diagnosis of Cowden syndrome, which is thought to affect 1 in 200 000 individuals in the population.

This report illustrates the importance of learning more about established phenotypes, clinical diagnostic criteria and the impact of panel testing. It explores challenges in identifying patients with unexpected mutations and the importance of recommending validated cancer screening. Reflection on such cases can be helpful in improving our understanding of panel testing, rare cancer susceptibilities and improving patient and family experiences.

#### P12.072

#### Telomeric length analysis in CRC tumors

**P. Mogollón Arroyo<sup>1,2</sup>, J. Pérez<sup>1</sup>, J. Perea<sup>2</sup>, J. García<sup>4</sup>, M. Urioste<sup>5</sup>, D. Rueda<sup>6</sup>, M. Arriba<sup>2</sup>, R. González-Sarmiento<sup>1,2,7</sup>;**

<sup>1</sup>Centro de Investigación del Cáncer, Salamanca, Spain, <sup>2</sup>Department of medicine, University of Salamanca, Salamanca, Spain, <sup>3</sup>Centre for biomedical research of 12 de Octubre University Hospital, Madrid, Spain, <sup>4</sup>Institute of Molecular and Cellular Biology of Cancer (IBMC), Salamanca, Spain, <sup>5</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Institute of Health Carlos III, Madrid, Spain, <sup>6</sup>Molecular biology laboratory, 12 de Octubre University Hospital, Madrid, Spain, <sup>7</sup>Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain.

Early-onset Colorectal Cancer (ECRC) represents a significant and increas-

sing proportion of Colorectal Cancer (CRC) and it is a heterogeneous entity that probably encompasses specific subclasses. It has been reported that shortening of telomere length is associated with an increased risk of cancer. Telomeres are the protective end-complexes of eukaryotic chromosomes since they are involved in the maintenance of normal chromosome structure and function. It has been demonstrated that telomerase is crucial for controlling telomere length, however, telomeres undergo a shortening with each cell division. In addition, it has been reported that telomeric shortening is associated with an early onset cancer.

Initially, we performed a cytogenetic study (CGH-Array) 46 tumors of late-onset CRC patients (over 70 years) and 45 tumors of early-onset CRC patients (under 45). We identified a group of tumors (mostly early-onset) that had a similar cytogenetic profile so the aim of our study was to analyze differences in telomeric length between tumors with the same cytogenetic profile (early and late-onset) versus tumors with a different profile.

Our results show that telomere length correlates with the cytogenetic profile in early-onset CRC tumors. Interestingly, there are not significant differences when compare the telomeric length of early-onset CRC tumors with the same cytogenetic profile versus late-onset CRC tumors.

These results reinforce the hypothesis that early-onset CRC cancer represents a specific subgroup of colon cancer.

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#### P12.073

#### Using of droplet digital PCR for tumor heterogeneity testing in gastrointestinal stromal tumors

**K. Jasek<sup>1</sup>, P. Szepe<sup>2</sup>, L. Plank<sup>2</sup>, Z. Lasabova<sup>1</sup>;**

<sup>1</sup>Biomedical Centre, Martin, Slovakia, <sup>2</sup>Department of Pathological Anatomy, Martin, Slovakia.

**Introduction:** Gastrointestinal stromal tumors (GISTs) are characterized by KIT/PDGFRa mutations. However, around 10-15% of all diagnosed GISTs are referred to as wild-type (WT) GISTs and V600E mutation in BRAF was in <5% of cases. The aim of this study was to confirm and quantify the presence of BRAF mutation using sensitive droplet digital PCR (ddPCR) in GISTs samples previously positive for V600E by allele-specific approach and/or dideoxysequencing.

**Material and methods:** DNA was extracted from 9 FFPE tissue section of V600E positive GISTs patients. Different amount of DNA – 130/100/10ng were used to prepare master mix with ddPCR Mut Assay BRAF and droplets were generated. Serial dilutions of V600E- positive RKO cell line DNA in wild-type DNA were used for calibration.

**Results:** The average number of droplets was 13696/PCR. The mutation could be detected in a dilution of 0.08%. The presence of V600E mutation in the 8 tested samples (positive by allele-specific PCR) was 1.9 % up to 0.34%. Sample positive for 1.9 % and 0.34% showed positivity and negativity by Sanger sequencing, respectively. One sample shows positivity below the limit of the detection of 0.06%, however was positive by allele-specific PCR. **Conclusions:** The BRAF mutation in GISTs occur in a very low number of cells, however, the detection of these mutations is particularly important in terms of propped treatment and targeted therapy. The method should be evaluated to higher sensitivity and used in order to improve the therapy opportunities of patients.

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#### P12.075

#### The excision of centromere as stabilization mechanism of dicentric chromosomes in bone marrow cells of two patients with acute myeloid leukemia

**I. Sarova<sup>1,2</sup>, J. Brezinova<sup>1</sup>, S. Ransdorfova<sup>1</sup>, Z. Zemanova<sup>2</sup>, L. Pavlistova<sup>2</sup>, K. Svobodova<sup>2</sup>, J. Cermak<sup>1</sup>, A. Jonasova<sup>3</sup>, K. Michalova<sup>1,2</sup>;**

<sup>1</sup>Institute of Hematology and Blood Transfusion, Prague, Czech Republic, <sup>2</sup>Center of Oncocytogenetics, IMBLD, General University Hospital and First Faculty of Medicine, Prague, Czech Republic, <sup>3</sup>1st Department of Internal Medicine of General University Hospital and 1st Faculty of Medicine, Prague, Czech Republic.

Dicentric chromosomes (DCs), have been observed in variety types of malignancies. Barbara McClintock (1939) first described their unstable behavior during cell division. The segregation of centromeres to opposite spindle poles can result in a cascade of chromosomal damages (the breakage-fusion-bridge cycle) and consequently in a complex unbalanced rearrangement of the karyotype. Three possible mechanisms of dicentric stabilization have been observed: epigenetic inactivation, reduction in the intercentromeric distance, and shortening/deletion of a centromere, resulting in a secondarily monocentric chromosome.

We describe two patients with acute myeloid leukemia and complex karyotype involved derivative chromosomes: der(11)(11pter→11q25::11q23→1

1q25::9p24→9p23::11q14→11q25::11q23→11q25::9p23→9p11::11q14.3→11q14.3::9q11→9qter) in one case and der(17)(17qter→17p11.2::11p11.2::11q11.2→11qter) accompanied by the separately localized centromere of chromosome 11, present as a small marker chromosome, in the second one. On the basis of molecular cytogenetic results (FISH, mFISH, mBAND), we inferred that the derivative chromosome der(11), respective der(17) were primary dicentric chromosomes, stabilized by the excision of centromere 9, respective 11.

In 2011, MacKinnon and Campbell first described centromere deletion in naturally occurring cancer chromosomes. Some studies suggest that the centromere excision is under-diagnosed, because centromere as a small marker chromosome can be usually detected by FISH only. Not in all cases, the centromere fragment is preserved as we present in patient 1. In conclusion, the centromere excision is an important stabilization mechanism of DCs however further analyses on the larger cohort of patients are needed to establish its true incidence.

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## P12.076

### DICER1 cancer predisposition syndrome: Expanding the phenotype

J. J. O'Byrne, M. Duff, A. J. Green, L. Bradley;

Department of Clinical Genetics, Dublin 12, Ireland.

**Introduction:** DICER1 is a cancer predisposition syndrome. Although germline mutations are known to increase the risk of many diverse, rare tumours such as pleuropulmonary blastoma and sertoli leydig cell tumours and multinodular goitre, the complete phenotype is still poorly understood. The variability in expression and the lack of penetrance data continues to make DICER1 a difficult condition to diagnose and counsel around. In this study a cohort of three families with known pathogenic mutations is presented which expands the known phenotype.

**Materials and Methods:** Clinical review and pedigree analysis of three families with a known DICER1 pathogenic mutation was conducted and the phenotypes tabulated.

#### Results:

Table: Familial DICER mutations and phenotypes

Family No	Mutation	Pathology in Family members with confirmed DICER mutation (age of onset - yrs)		Pathology in family members not yet tested for DICER mutation (age of onset - yrs)
		Cancer	Non Cancer	
1 (Irish/ Caribbean)	c.5003dupA; p.Asn1668fs (exon 25-3)	1 Bilateral SLCT (18/20)	1 MNG (50s)	1 Cervical Cancer (age unknown)
2 (UK)	c.5493G>A; p.Trp1831* (exon 27)	OSCT Gynandroblas- toma (15)	3 MNG (19,21,22) 1 "abnormal womb cells" (age unknown)	1 SLCT (17yrs) 1 Ovarian Cyst (6) 1 Bone Tumor (16) 1 Brain Tumor (40s)
3 (Irish)	c.2233C>T; p.Arg745* (exon 16)	ASK(6)		1 Avascular RMS(9) 1 Brain Cancer (1) 1 Renal Cancer (8) 1 Leukaemia (9) 1 MNG (35) 1 Lung Cyst (age unknown)

ASK - Anaplastic sarcoma of the kidney; MNG - multinodular goitre; OSCT - Ovarian Sex Chord Stromal Tumor; RMS- Rhabdomyosarcoma

#### Conclusions:

In this study:

- multinodular goitre and SLCT are confirmed as frequent phenotypes in people with DICER1 pathogenic mutations but no case of PPB was documented.
- rare or very rare phenotypes such as OSCST, gynandroblastomas, ASK were observed, suggesting these may occur more frequently than expected.
- the penetrance is estimated to ~70%, while literature suggests a low penetrance<sup>1</sup>.
- the DICER1 cancer predisposition syndrome phenotype is extended which may guide the development of, much needed, tumour screening recommendations.

**References:** Foulkes WD *et al.* Nat Rev Cancer. 2014; 14(10):662-72.

## P12.077

### Constitutional PTEN expression in women with early-onset breast cancer

M. G. Tedesco<sup>1</sup>, S. Martini<sup>1</sup>, R. Zuntini<sup>1,2</sup>, S. Miccoli<sup>1,2</sup>, L. Godino<sup>1,2</sup>, A. Belardinelli<sup>3</sup>, G. Lanzoni<sup>1</sup>, A. Vaisfeld<sup>1</sup>, M. Romagnoli<sup>1</sup>, D. Turchetti<sup>1,2</sup>;

<sup>1</sup>U.O.Genetica Medica, Policlinico S.Orsola-Malpighi, Bologna, Bologna, Italy, <sup>2</sup>Centro di Ricerca sui Tumori Ereditari, Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, Bologna, Italy, <sup>3</sup>Immunoematologia e medicina trasfusionale, Policlinico S.Orsola-Malpighi, Bologna, Italy.

Breast cancer (BC), the most common malignancy in women, mostly arises in peri-post menopausal age, while early age at onset may suggest genetic

susceptibility. However, germline mutations are identified only in a minority of patients, suggesting a role for different risk factors. Among those, we considered PTEN levels, which in mouse models were associated to BC risk. We assessed PTEN expression in 31 women with early-onset BC (26-35 years), who had tested negative for BRCA1/2 mutations, using q-real time PCR. Expression levels were compared to the mean level of 23 healthy female blood donors aged 50 or older. Patients were then subdivided into three groups: 13 patients under-expressing PTEN (<80%), 8 over-expressing PTEN (>125%) and 10 with expression similar to controls. If compared to patients with normal or lower levels, patients overexpressing PTEN had a more recent diagnosis (7,38 vs 8,10 vs 11,15 years, respectively), larger tumors (mean diameter: 21,7 mm vs 17,6 vs 18, respectively), with a T2 stage in 50% of the group compared to 22.2% and 16.7% in normal and low expression groups, respectively. These differences, however, did not reach the statistical significance; no other differences were detected between the groups. To explore the mechanisms underlying different PTEN levels, PTEN mutations and defect of methylation were analyzed, with no alterations detected.

Our findings seem to exclude that PTEN levels may be a relevant risk factor for BC occurrence, but might suggest the presence of regulatory mechanisms raising PTEN levels after BC diagnosis, especially for larger size tumors.

## P12.078

### The experience of EGFR-genotyping for the individualization in treatment of the patients with lung cancer in Federal State Budgetary Institution "RCRC named after N.N. Blokhin" of the Ministry of Health of the Russian Federation

O. Arkhipova, M. Filippova, A. Semyanikhina, T. Dubrovina, K. Laktionov, L. Lyubchenko; FSBI „Russian cancer research center named after N.N. Blokhin”, Moscow, Russian Federation.

**Introduction:** Activating somatic mutations in the EGFR gene can be detected in up to 30% cases of non-small-cell lung cancer (NSCLC). According to clinical guidelines EGFR-genotyping is a routine diagnostic procedure before prescribing tyrosine kinase inhibitors. Aside EGFR-testing the detection of ALK-*EML4* gene rearrangements which occur in 4-10% of all adenocarcinoma cases is recommended prior to initiating targeted therapy.

**Methods and materials:** EGFR-genotyping and ALK-testing of the DNA derived from the FFPE NSCLC samples were carried out. The specimens were collected from the 279 patients with NSCLC (179 women and 100 men). The median age at diagnosis was 57 years. Adenocarcinoma was diagnosed in 87%. DNA-testing was performed using Cobas EGFR Mutation Test Kit. To confirm the results Sanger sequencing was performed. ALK screening was carried out using the VENTANA anti-ALK (D5F3) assay.

**Results:** Mutations in the EGFR gene were detected in 28,5% of all tested samples. Mutations in exon 19 were found in 41%, L858R in 35%, T790M in 12%, G719C in 5% and an insertion in exon 20 in 7%. The combination of two activating mutations was revealed in two patients. We identified two ALK-positive samples.

**Conclusion:** Our data on the frequency and distribution of the mutations in the EGFR gene and ALK-rearrangements is comparable with the results previously reported. The cases with two activating but functionally different mutations confirm the heterogeneity of NSCLC but further research is necessary to determine the frequency of this phenomenon for a better understanding of the mechanisms of responsiveness to targeted therapy.

## P12.079

### Hereditary Breast and Ovarian Cancer Syndrome: Screening ERCC4 (FANCO) when BRCAs Mutations are not the Origin

C. Velázquez Pérez<sup>1</sup>, M. Durán Domínguez<sup>1</sup>, E. Esteban Cardeñosa<sup>1</sup>, M. Tascón Torres<sup>1</sup>, E. Lastra Aras<sup>2</sup>, G. Marcos García<sup>3</sup>, L. Hernández Sanz<sup>1</sup>, N. Martínez Martín<sup>1</sup>, M. Infante Sanz<sup>1</sup>;

<sup>1</sup>IBGM, Valladolid, Spain, <sup>2</sup>Servicio de Oncología, Complejo Hospitalario Burgos, Burgos, Spain, <sup>3</sup>Servicio de Oncología, Hospital Río Hortega, Valladolid, Spain.

ERCC4 is a versatile protein implicated in several DNA repair via: nucleotide excision repair (NER) pathway and Fanconi Anemia (FA) pathway. FA genes products are involved in DNA interstrand crosslink repair. Biallelic mutations in ERCC4 cause FA subtype Q whereas monoallelic mutations contribute to inherited risk of Hereditary Breast and Ovarian Cancer Syndrome (HBOC). In a clinical scope, via NER alterations conferred platinum sensitivity, the standard therapy for breast and ovarian cancer.

To investigate the role of ERCC4 in HBOC we screened this gene for mutations in 125 Spanish ovarian cancer patients or breast cancer cases with ovarian history in their pedigrees. All samples were negatives for BRCA mutations. Mutation screening was performed by HA-CAE and subsequently

Sanger Sequencing of altered pattern. Missense mutations were evaluated using CONDEL software that combines tools as SIFT, Polyphen and MutationAssessor.

Our study identified two novel variants (c.338+13A>G, c.1524 A>T) and eleven previously described (c.251C>T, c.974-7A>G, c.974-53delTG, c.1244G>A, c.1251T>A, c.1563C>G, c.1727G>C, c.1812-103G>A, c.1905-35T>C, c.1905-28G>A and c.2505T>C). In Silico analysis predicted c.1244 G>A and c.1727G>C as probably damaging.

We have identified ERCC4 inactivating mutations in 0.8% of our population, which is similar to other FA-BRCA genes. Hence, genetic testing of this gene should be considered in high risk HBOC families as far as ERCC4 mutations, that compromise DNA repair, could preserve sensitivity to continuous platinum therapy.

#### P12.080

##### Clinical and pathogenetic features of ETV6-related thrombocytopenia with predisposition to childhood ALL

F. Melazzini<sup>1</sup>, F. Palombo<sup>2</sup>, A. Baldiuni<sup>3</sup>, D. De Rocco<sup>4</sup>, C. Marconi<sup>2</sup>, P. Noris<sup>1</sup>, C. Gnan<sup>5</sup>, T. Pippucci<sup>2</sup>, V. Bozzi<sup>1</sup>, M. Faleschini<sup>4</sup>, C. A. Di Buduo<sup>6</sup>, C. Alfano<sup>7</sup>, M. Seri<sup>2</sup>, C. L. Baldiuni<sup>1</sup>, A. Pecci<sup>1</sup>, A. Savoia<sup>3,4</sup>.

<sup>1</sup>Department of Internal Medicine, University of Pavia-IRCCS Policlinico San Matteo Foundation, Pavia, Italy, <sup>2</sup>Department of Medical and Surgical Sciences, University of Bologna Medical Genetic Unit, Sant'Orsola Malpighi Hospital, Bologna, Italy,

<sup>3</sup>Laboratory of Biotechnology, IRCCS San Matteo Foundation, Pavia, Italy, <sup>4</sup>Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Trieste, Italy, <sup>5</sup>Department of Medical Sciences, University of Trieste, Trieste, Italy, <sup>6</sup>Department of Molecular Medicine, University of Pavia, Pavia, Italy, <sup>7</sup>Maurice Wohl Institute, King's College London, London, United Kingdom.

Inherited thrombocytopenias (ITs) are genetic disorders characterized by reduced blood platelet count. In addition to spontaneous bleedings, IT can be associated with other features, including risk of leukemias as in the ITs caused by mutations of RUNX1 and ANKRD26. Thanks to an international collaboration, we have recently identified another form of IT with an increased risk of hematological malignancies (ETV6-related thrombocytopenia; ETV6RT) that is caused by mutations of ETV6, a relatively well-known tumor-suppressor gene (Noetzli et al., 2015). We searched for ETV6 mutations in a series of 130 consecutive propositi with ITs of unknown origin and found 5 causative variants in 7 of them. Although the study of family members revealed that degree of thrombocytopenia and bleeding tendency were mild, 4 patients had childhood B-cell acute lymphoblastic leukemia (ALL), confirming that ETV6-RT is a ALL predisposition syndrome. In vitro studies revealed that patient megakaryocytes have defective maturation and proplatelet formation, while platelet have reduced ability to spread on fibrinogen; however clinical and laboratory findings did not identify any peculiar defect that can be used to suspect this disorder and also platelet size (usually enlarged in ITs) was normal. For these reasons, we suggest that mutation screening of ETV6 is performed in subjects with dominant ITs and normally sized platelets.

#### P12.081

##### Polymorphisms associated with everolimus pharmacokinetics, toxicity and survival in metastatic breast cancer

M. Apellániz-Ruiz<sup>1</sup>, T. Pascual-Martínez<sup>2</sup>, C. Pernaut<sup>3</sup>, C. Cueto-Felgueroso<sup>4</sup>, P. Villalba<sup>4</sup>, C. Álvarez<sup>4</sup>, L. Manso<sup>5</sup>, L. Inglada-Pérez<sup>1,5</sup>, M. Robledo<sup>1,5</sup>, C. Rodríguez-Antón<sup>1,5</sup>, E. Ciruelos<sup>3</sup>.

<sup>1</sup>Spanish National Cancer Research Centre (CNIO), Madrid, Spain, <sup>2</sup>Medical Oncology Department, La Princesa University Hospital, Madrid, Spain, <sup>3</sup>Medical Oncology Department, 12 de Octubre University Hospital, Madrid, Spain, <sup>4</sup>Biochemistry Department, 12 de Octubre University Hospital, Madrid, Spain, <sup>5</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

**Background:** Metastatic breast cancer (MBC) progressing after endocrine therapy frequently activates mTOR pathway. The BOLERO-2 trial showed that everolimus-exemestane achieves increased progression free survival (PFS) compared with exemestane. However, there is great inter-patient variability in toxicity and response to exemestane-everolimus treatment. The objective of this study was to explore the implication of single nucleotide polymorphisms (SNPs) on outcomes from this treatment through a pharmacogenetic analysis.

**Patients and Methods:** Blood was collected from 90 postmenopausal women with hormone receptor-positive, HER2-negative MBC treated with exemestane-everolimus following progression after prior treatment with a non-steroidal aromatase inhibitor. Everolimus pharmacokinetics was measured in 37 patients. Twelve SNPs in genes involved in everolimus pharmacokinetics and pharmacodynamics were genotyped and associations assessed with drug plasma levels, clinically relevant toxicities (non-infectious pneumonitis, mucositis, hyperglycemia and hematological toxicities), dose reductions

or treatment suspensions due to toxicity, progression free survival (PFS) and overall survival.

**Results:** We found that CYP3A4 rs35599367 variant (CYP3A4\*22 allele) carriers had higher everolimus blood concentration compared to wild type patients (P=0.019). ABCB1 rs1045642 was associated with risk of mucositis (P=0.031), while PIK3R1 rs10515074 and RAPTOR rs9906827 were associated with hyperglycemia and non-infectious pneumonitis (P=0.016 and 0.024, respectively). Furthermore, RAPTOR rs9906827 was associated with PFS (P=0.006).

**Conclusions:** CYP3A4\*22 allele influenced plasma concentration of everolimus and several SNPs in mTOR pathway genes were associated with treatment toxicities and prognosis. These results require replication, but suggest that germline variation could influence everolimus outcomes in MBC.

#### P12.082

##### Mutational status of EZH2 and CD79B hot spots in mature B-cell non-Hodgkin's lymphomas: novel CD79B variations have been revealed

G. Cetin<sup>1</sup>, I. Baris<sup>2</sup>, V. Caner<sup>1</sup>, B. Sarikepe<sup>1</sup>, N. Sen Turk<sup>3</sup>, E. Tepeli<sup>1</sup>, S. Hacioglu<sup>4</sup>, I. Sarı<sup>1</sup>, G. Bagci<sup>1</sup>, A. Keskin<sup>4</sup>.

<sup>1</sup>Pamukkale University School of Medicine Department of Medical Genetics, Denizli, Turkey, <sup>2</sup>Pamukkale University School of Medicine Department of Medical Biology, Denizli, Turkey, <sup>3</sup>Pamukkale University School of Medicine Department of Medical Pathology, Denizli, Turkey, <sup>4</sup>Pamukkale University School of Medicine Department of Hematology, Denizli, Turkey.

**OBJECTIVE:** We aimed to determine the hot spot mutational frequencies of Enhancer of Zeste homolog 2 (EZH2) and cluster of differentiation 79B (CD79B) genes in a cohort of mature B-cell non-Hodgkin's lymphomas.

**MATERIALS AND METHODS:** DNA samples from formalin-fixed and paraffin embedded (FFPE) tissues from a total of 37 patients with mature B-cell non-Hodgkin lymphomas were included in the study. Molecular genetic analysis was performed by direct sequencing of the DNA samples.

**RESULTS:** We analyzed FFPE tumor tissue samples from 17 female and 20 male patients with a median age of 63.7 years at the time of diagnosis. None of the patients had previously reported hot spot mutations in EZH2 and CD79B, but previously unreported single nucleotide variations of CD79B were present in nine patients. rs779833118 was the most frequent variation (7/37 patients, 18.9%). A non-synonymous variation rs757407417, which could have a potentially damaging outcome, was detected in two patients.

**CONCLUSIONS:** None of the patients had well-known hot spot mutations in EZH2 and CD79B. However, we detected novel CD79B variations in mature B-cell non-Hodgkin's lymphoma patients.

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#### P12.083

##### Extracolonic tumors as presenting symptoms of FAP in probands with a family history of FAP

C. Vinkler<sup>1</sup>, K. I. Dayan<sup>2</sup>, A. Singer<sup>3</sup>, R. Berger<sup>4</sup>, D. Lev<sup>1</sup>.

<sup>1</sup>Institute of Clinical Genetics Wolfson Medical Ctr, Holon, Israel, <sup>2</sup>Dpt of Surgery, Wolfson Med Ctr, Holon, Israel, <sup>3</sup>Department of Community Genetics Ministry of Health, Jerusalem, Israel, <sup>4</sup>Institute of Oncology Wolfson Medical Ctr, Holon, Israel.

Sporadic desmoid tumors is estimated at 1 to 2 per million, whereas more than 15% of patients suffering from familial adenomatous polyposis (FAP) develop desmoid tumors.

Nonmedullary Thyroid Cancer (NMTC) has an incidence of 12.1:100,000. 5-10% are familial cases caused by germline mutations in specific genes. A small number are associated with known syndromes. Rarely NMTC is an extracolonic manifestation of FAP.

FAP is a colon cancer susceptibility syndrome in which hundreds to thousands of colonic polyps develop. By 35y, 95% of individuals with FAP have polyps.

We report two patients with FAP presenting first with extracolonic manifestation.

A 33 y old woman with thyroid goiter whose sister and her father have NMTC. Her 35y old brother had GI bleeding and three adenomatous polyps removed on colonoscopy. Sequencing of APC gene in the brother revealed a frame shift mutation p.Arg332fs\*.

A 53-year-old male presented first with intestinal obstruction. Two small adenomatous polyps were found during colonoscopy. A large peritoneal desmoid tumor was removed and symptoms were alleviated. FAP has been diagnosed previously in other family members. He was found to carry a frameshift mutation in the APC gene p.Ser299Cysfs\*.

This unusual presentation demonstrates the clinical importance of a comprehensive evaluation of familial cancer cases. It also shows the intrafamilial variability in the phenotypic manifestations of the familial mutations. It may

be postulated that difference in genetic background along with environmental factors may be involved in modifying the phenotypic expression in these cases.

#### P12.085

#### Identification of susceptibility genes to define the genetic basis of familial testicular cancer by whole exome sequencing

**B. Paumard Hernandez**<sup>1</sup>, M. Urioste<sup>1,2</sup>, E. Gonzalez Billalabeitia<sup>3</sup>, I. Marquez-Rodas<sup>4</sup>, J. Maroto<sup>5</sup>, G. Anguera Palacios<sup>6</sup>, N. Romero-Laorden<sup>7</sup>, J. Triviño<sup>8</sup>, H. Tejero<sup>9</sup>, J. Benítez<sup>9,2</sup>,

<sup>1</sup>Spanish National Cancer Research Center (CNIO), Madrid, Spain, <sup>2</sup>Center for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain, <sup>3</sup>Hospital Universitario Morales Meseguer, Murcia, Spain, <sup>4</sup>Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, <sup>5</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>6</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>7</sup>Centro Integral Oncológico Clara Campal, Madrid, Spain, <sup>8</sup>Sistemas Genómicos, Valencia, Spain, <sup>9</sup>Spanish National Cancer Research Center (CNIO), Madrid, Spain.

**Introduction:** Testicular cancer represents ~1-1.5% of male tumors, with a peak incidence between 15 and 45 years. Human Testicular germ cell tumors (TGCT) have a strong genetic component, familial aggregation is observed in ~1% of cases, although no high penetrance genes have been identified so far. The aim is the identification of high-/moderate susceptibility genes by WES technique.

**Materials and Methods:** Currently, 20 families with at least two affected members were selected, from them, 8 have already been sequenced using a HiSeq2000. Data was analyzed according to the specific pipeline described by our group based on different filters. Considering the recent findings in the genetic background of the TGCTs and our preliminary results, we are working on the hypothesis of a polygenic model of inheritance, making a family-based association test in parallel with a population-based association test with the FB-SKAT test, to evaluate the level of additive effect that our variants could have with the familial aggregation of the disease.

**Results:** 87 variants were identified in candidate genes, whose functions have been implicated in pathways involving male germ cell development, spermatogenesis, microtubule assembly, DNA damage response, steroid hormone signaling or telomerase function. From them, 65 were validated by Sanger sequencing and segregation analysis performed.

Nine, seven and one variants are shared between 2, 3 and 4 different families, respectively.

**Conclusion:** Our preliminary results show that a number of moderate-low susceptibility alleles from specific pathways could be involved in this type of familial tumors.

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#### P12.086

#### Identification of germline FAN1 variants in MSH2-deficient Lynch-like syndrome patients

**G. Vargas**<sup>1</sup>, E. Dámaso<sup>1</sup>, M. Navarro<sup>1</sup>, T. Pons<sup>2</sup>, L. Mina<sup>3</sup>, A. Fernández<sup>1</sup>, L. Feliubadaló<sup>1</sup>, A. Solanes<sup>4</sup>, S. Iglesias<sup>5</sup>, Á. Velasco<sup>6</sup>, J. Balmáñez<sup>6</sup>, T. Ramón y Cajal<sup>7</sup>, A. Valencia<sup>2</sup>, J. Brunet<sup>6</sup>, J. Surrallés<sup>8</sup>, C. Lázaro<sup>9</sup>, L. Valle<sup>10</sup>, M. Pineda<sup>11</sup>, **G. Capellá**<sup>12</sup>;

<sup>1</sup>Institut d'Investigació Biomèdica de Bellvitge-Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain, <sup>2</sup>Spanish National Cancer Research Center (CNIO), Madrid, Spain, <sup>3</sup>Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, Spain, <sup>4</sup>Institut d'Investigació Germans Trias i Pujol-Institut Català d'Oncologia, Badalona, Spain, <sup>5</sup>Institut d'Investigació Biomèdica de Girona Doctor Josep Trueta-Institut Català d'Oncologia, Girona, Spain, <sup>6</sup>University Hospital Vall d'Hebron and Vall d'Hebron Institute of Oncology, Barcelona, Spain, <sup>7</sup>Santa Creu i Sant Pau Hospital, Barcelona, Spain.

**Introduction:** In about 55% of individuals harboring mismatch repair (MMR) deficient tumors, germline mutations or somatic methylation in MMR genes are not identified, being referred as Lynch-like syndrome (LLS) patients. Recently FAN1 germline mutations have been associated to MMR proficient colorectal cancer (CRC) and pancreatic cancer predisposition. The aim of this study was to determine whether germline FAN1 play also a role in LLS. **Patients and methods:** Germline analysis of FAN1 was performed in 30 LLS unrelated individuals showing MSH2 loss of expression in tumors. Pathogenicity assessment of identified variants was performed using computational and cosegregation analyses.

**Results:** We identified three rare missense variants in 3 unrelated LLS patients (10% of the studied sample). Two of the 3 identified variants, c.434G>A [p.(R145H)] and c.1129C>T [p.(R377W)], cosegregated with colorectal cancer-affected relatives. The remaining variant, c.1856T>A (p.M619K), for which no cosegregation data was available, was classified as likely pathogenic based on functional and computational analyses.

**Conclusion:** The obtained results suggest the involvement of the FAN1 gene in MSH2-deficient LLS.

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#### P12.087

#### FANCM: A novel breast cancer gene subjected to an international validation study

**G. Neidhardt**<sup>1,2,3</sup>, J. Hauke<sup>1,2,3</sup>, S. Heilmann-Heimbach<sup>4,5</sup>, H. Hellebrand<sup>6</sup>, H. Surowy<sup>7</sup>, K. Klaschik<sup>1,2,3</sup>, E. Honisch<sup>8</sup>, A. Gehrig<sup>9</sup>, C. Sutter<sup>10</sup>, A. Rump<sup>11</sup>, N. Bogdanova-Markov<sup>12</sup>, P. Bugert<sup>13</sup>, E. Mangold<sup>14</sup>, D. Steinemann<sup>14</sup>, A. Ramirez<sup>15</sup>, N. Ditsch<sup>6</sup>, N. Arnold<sup>16</sup>, D. Niederacher<sup>8</sup>, B. Burwinkel<sup>7</sup>, H. Thiele<sup>17</sup>, J. Altmüller<sup>17,18</sup>, P. Nürnberg<sup>17,18,19</sup>, C. Engel<sup>20</sup>, B. Wappenschmidt<sup>1,2,3</sup>, K. Rhiem<sup>1,2,3</sup>, A. Meindl<sup>6</sup>, R. K. Schmutzler<sup>1,2,3</sup>, E. Hahnen<sup>1,2,3</sup>,

<sup>1</sup>Center for Familial Breast and Ovarian Cancer, Cologne, Germany, <sup>2</sup>Center for Integrated Oncology (CIO), Cologne, Germany, <sup>3</sup>Center for Molecular Medicine Cologne (CMMC), Cologne, Germany, <sup>4</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>5</sup>Life&Brain Center, Bonn, Germany, <sup>6</sup>Department of Gynaecology and Obstetrics, Klinikum rechts der Isar at the Technical University, Munich, Germany, <sup>7</sup>Molecular Epidemiology, German Cancer Research Center (DKFZ) and Molecular Biology of Breast Cancer, Heidelberg University Women's Hospital, Heidelberg, Germany, <sup>8</sup>Department of Obstetrics and Gynecology, University Medical Center Düsseldorf, Heinrich-Heine-University, Düsseldorf, Germany, <sup>9</sup>Department of Human Genetics, University Würzburg, Biozentrum, Würzburg, Germany, <sup>10</sup>Institute of Human Genetics, Ruprecht-Karls University, Heidelberg, Germany, <sup>11</sup>Institute for Clinical Genetics, Technische Universität Dresden, Dresden, Germany, <sup>12</sup>Institute of Human Genetics, University of Münster, Münster, Germany, <sup>13</sup>Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, University of Heidelberg, German Red Cross Blood Service Baden-Württemberg-Hessia, Mannheim, Germany, <sup>14</sup>Institute of Pathology, Hannover Medical School, Hannover, Germany, <sup>15</sup>Department of Psychiatry and Psychotherapy of Bonn University Hospital, Bonn, Germany, <sup>16</sup>Division of Oncology, Department of Gynaecology and Obstetrics, University Hospital Schleswig-Holstein, Christian-Albrechts-University, Kiel, Germany, <sup>17</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>18</sup>Institute of Human Genetics, University of Cologne, Cologne, Germany, <sup>19</sup>Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany, <sup>20</sup>Institute for Medical Inform

Recently, Kiiski et al. reported a significant association between the truncating FANCM mutation p.Gln1701Ter and BC risk in the Finish population (OR 1.86, 95% CI = 1.26-2.75; P = 0.0018). By analyzing the exomes of 24 BRCA1/2-negative index cases from high-risk BC/OC families of German origin, we also found the p.Gln1701Ter mutation. Mutational analysis of the FANCM gene by NGS in 2,211 familial BRCA1/2-negative BC and/or OC index cases identified 7 different truncating alterations in 26 cases (1x p.Gln156Ter, 12x p.Gln1701Ter, 1x p.Arg1030Ter, 1x p.Arg185Glufs, 5x p.Arg1931Ter, 5x p.Arg658Ter, 1x p.Val1095Tyrfs; 26/2,211 CF=1.176%). We were able to calculate an OR of 2.24 (95%CI=1.26-3.95 p=0.004) by comparing our findings to the FANCM mutation load in 5,335 control individuals of which 28 were tested positive (28/5,335 CF=0.525%). In an extended cohort of 6,117 familial BRCA1/2-negative BC and/or OC index cases of German origin we genotyped the p.Gln1701Ter mutation 20 times (20/6,117, CF=0.327%) and 14 times in 9,786 control individuals (14/9,786, CF=0.143%), resulting in an OR of 2.29 (95%CI=1.10-4.77 p=0.013). The mean age at first BC diagnosis of individuals carrying truncating FANCM alterations was 49 years with predominantly ER+, PR+, HER2-tumours of grade 2. Due to the apparently low FANCM mutation frequency, a large collaborative international study group was founded (FANCM study group), including research groups from Finland, Germany, Italy and the USA to quantify the risk for BC and possibly other cancer entities associated with deleterious FANCM alterations.

#### P12.088

#### Modelling the Fanconi anemia/BRCA pathway by TALEN and CRISPR/Cas9 engineered nucleases

**M. Aza**<sup>1,2</sup>, M. Bogliolo<sup>1,2</sup>, M. Marín<sup>1,2</sup>, L. Mina<sup>1,2</sup>, Á. Muñoz<sup>1</sup>, M. Ramírez<sup>1,2</sup>, **J. Surrallés**<sup>1,2</sup>,

<sup>1</sup>Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain.

**Introduction:** Fanconi anemia (FA) is a rare genome instability disorder clinically characterized by developmental abnormalities, high predisposition to cancer and bone marrow failure. Many of the available cell lines from FA anemia patients are difficult to manipulate and are not isogenic.

**Objectives:** Due to current limitations of FA cell models, we decided to knock-out (KO) genes implicated in the FA/BRCA pathway (FANCA, FANCD1, FANCIQ and FAN1) in the genetically amenable human HEK293T cell line.

**Methods:** We used TALENs and CRISPR/Cas9 for gene KO. Specific gene disruption was proven by genetic complementation with the corresponding wildtype gene using lentiviral vectors or transient transfection.

**Results:** We successfully generated FANCA, FANCD1, FANCIQ and FAN1 KO HEK293T cells using engineered nucleases. All these cells are sensitive to DNA crosslinking agents, thus mimicking cells from patients with defects in the FA/BRCA pathway. Moreover, all FA genes KO cells get blocked in the G2/M phase upon treatment with DNA crosslinkers, a typical FA cellular phenotype. Finally, these FA cells reproduce gene-specific defects: FANCA-/ cells have impaired FANCIQ monoubiquitination, FANCD1/- cells are unab-

le to form Rad51 foci after irradiation and are sensitive to PARP inhibitors, and FANCQ-/- cells are sensitive to UV-light radiation.

**Conclusion:** Genome editing with targeted engineered nucleases is a valuable tool to generate isogenic and genetically amenable FA cell models. The generated FA/BRCA KO isogenic cells will facilitate translational studies including the analysis of pathogenicity of variants of unknown significance, large-scale drug screenings or proteomic projects to identify new FA/BRCA pathway genes.

#### P12.089

#### Germline TP53 mutation in a patient with fibrolamellar carcinoma: new association to Li-Fraumeni Syndrome?

R. C. Andrade<sup>1</sup>, M. A. F. D. Lima<sup>2</sup>, P. A. S. Faria<sup>3</sup>, F. R. Vargas<sup>1,4,5</sup>,

<sup>1</sup>Genetics Division, Instituto Nacional de Câncer, Rio de Janeiro, Brazil, <sup>2</sup>Programa de Internato em Genética, Universidade UnigranRio, Rio de Janeiro, Brazil, <sup>3</sup>Pathology Division, Instituto Nacional de Câncer, Rio de Janeiro, Brazil, <sup>4</sup>Genetics and Molecular Biology Department, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil, <sup>5</sup>Birth Defects Epidemiology Laboratory, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Li-Fraumeni (LFS) syndrome is a rare hereditary cancer predisposition syndrome associated with germline mutations in *TP53* gene. We performed *TP53* mutation screening in a cohort of 14 probands whose family cancer histories presented at least one LFS core cancer before age 45, and a second cancer of any type before age 45, irrespective of degree of kinship. These features are reminiscent of LFS, yet not compatible with LFS or Li-Fraumeni like (LFL) published criteria. A pathogenic *TP53* mutation was identified in 1/14 probands (7.1%), a 14 year-old female diagnosed with fibrolamellar carcinoma (FLC). FLC is a rare variant of hepatocellular carcinoma with distinct molecular features. The proband is a heterozygote carrier of the *TP53* c.467G>A (p.Arg156His) in exon 5, and her mother is an asymptomatic carrier. Analysis of tumor DNA disclosed an additional alteration (c.461G>A; p.Gly154Asp) in *TP53*. This is the first case of a proband with FLC exhibiting a germline and a somatic *TP53* pathogenic mutation, possibly acting as driver alterations. This report adds information to the molecular etiology of FLC, and to the continuously growing spectrum of tumors related to LFS. Importantly, probands with family history reminiscent of LFS/LFL should be considered for *TP53* screening.

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#### P12.090

#### Cloud-based informatics enables the design and analysis of massively multiplex custom gene fusion panels for next-generation sequencing on FFPE RNA samples.

R. Gottumukkala, E. Ballesteros, H. Breu, Y. Lou, S. Myrand, M. Hogan, K. Bramlett, G. Liu, S. Sadis, F. Hyland, F. Hernandez-Guzman;

Thermo Fisher, South San Francisco, CA, United States.

Gene fusions, a combination of two genes, are caused by structural DNA rearrangements. Many gene fusions are strong driver mutations in neoplasia, and are important in understanding interaction with targeted therapy and risk stratification.

Massively multiplex Ampliseq gene fusion assays with next-generation sequencing enable enrichment of fusion transcripts using 10 ng of RNA extracted from FFPE samples, with sensitive detection of particular fusion isoforms for defined gene pairs. Ion Torrent sequencing reveals the full sequence of the gene fusion.

We developed cloud-based software to enable the design of a custom Ampliseq gene fusion panel, with up to 1,000 fusions and gene expression assays. We extensively mined the scientific literature and the COSMIC database for fusions. We rigorously curated this data, including correction of reported sequence to obtain genomic coordinates, breakpoints, exon junctions, and selected wet lab testing. We created a database containing over 1000 high quality fusion isoforms, including 70 ALK, 60 RET, 26 ROS1, and 21 NTRK1 fusions. We designed multiplexable Ampliseq primers for all fusions.

We developed cloud-based analysis software to analyze sequencing data, utilizing the rich annotation information. The reads are mapped to a custom fusion reference sequence; an optimized algorithm selects confidently mapped reads using overlap and breakpoint information, and sensitively detects gene fusions. Software QC steps for total number of mapped reads, number of reads for gene expression controls, and elimination of cross-talk artifacts result in a highly sensitive and specific detection of fusions, with LOD below 1%. Fusion results can be viewed, annotated, exported.

#### P12.092

#### Genetic susceptibility to gastric cancer: Immunohistochemical characterization of genes significantly associated with gastric cancer

A. V. Sanchez Romero<sup>1</sup>, N. García Lozano<sup>1</sup>, M. Paúles Villar<sup>2</sup>, L. Luján Barroso<sup>1</sup>, M. Galán Guzmán<sup>1</sup>, N. Sala Serra<sup>1</sup>;

<sup>1</sup>Catalan Institute of Oncology-Bellvitge Institute for Biomedical Research (ICO-IDIBELL), Hospital de Llobregat, Barcelona, Spain, <sup>2</sup>University Hospital of Bellvitge-IDIBELL, Hospital de Llobregat, Barcelona, Spain.

**Introduction:** From previous studies, we confirmed the association between gastric cancer (GC) in Europeans and variants in different genes, of which *MUC1*, *TFF1*, *NQO1* and *PTPN11* were selected for further characterization of their role in gastric carcinogenesis.

**Objectives:** To analyze the immunohistochemical (IHC) expression of these four genes in paired samples of tumor-normal gastric mucosa, according to the genotypes of their GC associated variants.

**Methods:** IHC staining was performed on tissue slides of 100 pairs of FFPE tissue blocks of tumor and adjacent non-tumor gastric mucosa from 100 patients with GC. Samples were grouped in three categories according to the percentage of IHC stained cells in gastric mucosa: 0-10%, >10-50% and >50-100%. The kappa statistic was used to evaluate the agreement between tumor and paired non-tumor tissues. The Fisher exact test was used to analyze differences between the histological subtypes of gastric cancer in tumor samples. Association between IHC expression of *TFF1*, *MUC1* and *NQO1* and *TFF1* rs2839488 and rs9976977, *MUC1* rs3814316 and rs4072037 and *NQO1* rs7359387, was analyzed by logistic regression.

**Results:** *TFF1* was significantly decreased or absent in tumor mucosa. *MUC1* and *NQO1* were expressed in both normal and tumor mucosa, particularly of the intestinal-type, but expression decreased in tumor samples. *PTPN11* expression was absent in 95% of the samples. None of the analyzed genetic variants was associated with the IHC expression of their genes.

**Conclusion:** The genotype of the analyzed GC-associated genetic variants does not contribute to the IHC expression of their genes.

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#### P12.093

#### Profiling of mutations in gastric cancer

K. I. Kirillova<sup>1,2</sup>, V. V. Strelnikov<sup>1,2</sup>, M. V. Nemtsova<sup>1,2</sup>, I. I. Bykov<sup>4</sup>, A. S. Tanas<sup>1,2</sup>;

<sup>1</sup>Research Centre for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation, <sup>3</sup>Russian Medical Academy for Postgraduate Education, Moscow, Russian Federation, <sup>4</sup>I.M.Sеченov First Moscow State Medical University, Moscow, Russian Federation.

**Introduction:** Gastric cancer (GC) is one of the most common oncological diseases with high morbidity and mortality. Genetic factors may increase the risk of developing GC. The aims of this study are to identify new genes involved in the development of GC and to characterize mutations in these genes. **Materials and methods:** The study included GC 52 patients aged 35-59 years, who had given the tumor material, normal gastric tissue and venous blood. Search for mutations in 52 cancer related genes was carried out by high-throughput parallel DNA sequencing. For the validation of the detected mutations we used Sanger sequencing. Determination of germline or somatic nature of the identified mutations was done by DNA sequencing of normal tissues of the same patients.

**Results:** Mutations were found in 22 out of 52 genes. We show that diffuse and intestinal GC types are distinguished by the spectra of somatic mutations: somatic mutations in the *CDH1* gene are more common in the diffuse type ( $p=0.04$ ) and mutations in the *TP53* gene are more common in the intestinal type of GC ( $p=0.04$ ). Germline mutations in patients with GC were for the first time detected in the genes *MET* (p.N375S, p.R970C), *RB1* (p.H686N), *EGFR* (p.V292M), *JAK3* (p.V722I), *APC* (p.I1289K), *ATM* (p.F858L), *CDKN2A* (p.D86N). New mutations were discovered in the *CDH1* gene (c.-71C>G, p.K182N, p.T303P, p.S838G, c.1320+2T>G).

**Conclusions:** Our results suggest that germline mutations in genes other than *CDH1* might contribute to GC development.

#### P12.094

#### MAP3K6 expression and methylation profile in sporadic Gastric Cancer

A. S. Valente<sup>1</sup>, P. Oliveira<sup>1</sup>, P. Ferreira<sup>1</sup>, J. Carvalho<sup>1</sup>, H. Pinheiro<sup>1</sup>, C. Oliveira<sup>1,2</sup>;

<sup>1</sup>Ipatimup/i3S, Porto, Portugal, <sup>2</sup>Medical Faculty of the University of Porto, Porto, Portugal.

**Introduction:** Every year, >1 million new Gastric Cancer (GC) cases are diagnosed and around 700.000 GC-related deaths are reported. Despite all efforts, much remains to be known on the molecular events involved. Important lessons can be learned from studies of familial cases as the genes and mechanisms are frequently shared with those found in the sporadic setting. Recently, we described MAP3K6 germline mutations in hereditary GC. Somatic hypermethylation of an intragenic CpG island, overlapping a DNase-

hypersensitive site predicted to harbour a promoter-associated regulatory element, has been presented as a putative second-hit. The aim of the present work was to evaluate the relevance of MAP3K6 in sporadic GC. **Materials and Methods:** MAP3K6 expression was evaluated by qRT-PCR in RNA from sporadic GC cases and corresponding adjacent normal mucosa. Methylation profile of MAP3K6 promoter and gene-body was assessed by RRBS. **Results:**

Overall, MAP3K6 was consistently downregulated in GC samples in comparison to corresponding adjacent mucosa. Promoter hypomethylation was a common event in tumours, but the methylation profile at the intragenic CpG island was unchanged compared to normal. **Conclusions:** MAP3K6 downregulation seems to be a common event in GC, correlating with promoter hypomethylation. These observations suggest the existence of a repressor binding site within MAP3K6 promoter, which becomes active upon demethylation. Further studies are warranted to corroborate these findings as well as to disclose the role of MAP3K6 in gastric carcinogenesis.

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#### P12.095

##### The tumorigenic pathways of MSI and CIN sporadic gastric cancers

J. Carvalho<sup>1,2</sup>, P. Ferreira<sup>1,2</sup>, P. Oliveira<sup>1,2</sup>, G. M. Almeida<sup>1,2</sup>, H. Pinheiro<sup>1,2</sup>, S. Rocha<sup>1,2</sup>, D. Ferreira<sup>1,2</sup>, A. S. Valente<sup>1,2</sup>, M. Santos<sup>1,2,3</sup>, F. Carneiro<sup>1,2,4</sup>, C. Oliveira<sup>1,2,3,4</sup>

<sup>1</sup>Expression Regulation in Cancer Group, IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal, <sup>2</sup>Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, <sup>3</sup>Department of Medical Sciences and Institute of Biomedicine – iBIMED, University of Aveiro, Aveiro, Portugal, <sup>4</sup>Medical Faculty of the University of Porto, Porto, Portugal.

**Introduction:** Gastric cancer (GC) kills ~700.000 people per year and 5-year overall survival is below 25%, due to late diagnosis and lack of effective therapies. Surgery is the single curative treatment, however helpful only for early-stage tumors. Targeted therapies are just starting to be applied with limited success. Recently, NGS technology provided the spectrum of somatic alterations in several GC series but its usefulness for decision-making in clinical practice is still scarce. We aimed at characterizing two GC molecular subgroups and predict targetable molecular signatures, which may guide better therapy selection strategies. **Material and Methods:** Fifty GC tumour/normal pairs either showing Chromosomal Instability (CIN=25) and bad prognosis, or Microsatellite Instability (MSI=25) and better prognosis, were analyzed by Whole genome and Methylome sequencing and integrative bioinformatics. Data was validated in independent TCGA datasets.

**Results:** MSI tumours displayed hypermethylated tumour suppressor genes and activating point mutations in oncogenes. CIN tumours displayed gene amplification at oncogenes and drug-resistance genes and hypomethylation on oncogene promoters. The same type of differential mechanisms was found for Chromatin remodeling, and Cell cycle control, but Splicing factors and RNA-binding proteins were affected similarly in both subgroups. Despite differences in mechanisms, genes encoding targetable oncogenic signalling proteins were similarly affected in both subgroups. **Conclusions:** These data demonstrate that most tumors present similar tumorigenic pathways independently of the molecular subgroup, and that differences in prognosis and drug response are likely related with the action of modifier proteins.

#### P12.096

##### Gene expression analysis of Gastric cancer Patients from Saudi Arabian Population

F. Bibi<sup>1</sup>, M. I. Nasser<sup>2</sup>, S. Akhtar<sup>2</sup>, E. I. Azhar<sup>1,2</sup>, M. Yasir<sup>4</sup>, A. A. Jiman-Fatani<sup>5</sup>, A. Sawan<sup>6</sup>; <sup>1</sup>Special Infectious Agents Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia, <sup>2</sup>Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia, <sup>3</sup>Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, <sup>4</sup>Special Infectious Agents Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia, <sup>5</sup>Department of Medical Microbiology and Parasitology, Faculty of Medicine, King, Jeddah, Saudi Arabia, <sup>6</sup>Department of Anatomical Pathology, Faculty of Medicine, King, Jeddah, Saudi Arabia.

The role of small non-coding microRNAs (miRNAs) in several types of cancer has been evident. However, its expression has not been studied in Saudi population in gastric cancer (GC) patients. First time this study was conducted to identify miRNAs that are differentially expressed in gastric cancer patients compared with normal controls using miRNA microarray analysis and validated the results by Real-time quantitative PCR (qPCR). Our objective was to investigate the role of microRNAs in GC patients from Saudi population using formalin-fixed paraffin-embedded (FFPE) tissues of 34 samples from GC patients and 15 from normal control. We obtained expression data of 1082 expressed genes, from cancer tissues and noncancerous tissues (48 samples in total). Where 129 genes are up-regulated ( $P > 0.05$ ) and 953 genes ( $P > 0.05$ ) are down-regulated in 45 FFPE tissue samples. After candidate miRNAs were selected, qPCR further confirmed that four miRNAs (hsa-miR-361-5p, hsa-miR-3687, hsa-miR-3613-3p, hsa-miR-1263) were significantly aberrant in GC tissues compared to the normal gastric tissues. In this study we provide miRNAs profile of GC where many miRNAs showed aberrant expression, suggesting the involvement of these genes in the development and progression of gastric cancer. Taken together, the results provided potential roles of these candidates' miRNAs in the diagnosis, prognosis biomarkers, or therapy targets of gastric cancer in Saudi population.

#### P12.097

##### Stomach Specific eQTLs represent Risk Factors for the Development of Gastric Carcinoma

T. Hess<sup>1,2</sup>, U. Gyvyyte<sup>3</sup>, J. Becker<sup>1,2</sup>, S. Heinrichs<sup>1,2</sup>, S. Lindenberg<sup>2</sup>, M. Leja<sup>4</sup>, M. Venerito<sup>5</sup>, J. Kupcinskas<sup>3</sup>, J. Schumacher<sup>1</sup>

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life & Brain Center, Bonn, Germany, <sup>3</sup>Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas, Lithuania, <sup>4</sup>Digestive Diseases Center GASTRO, Riga East University Hospital, University of Latvia, Riga, Latvia, <sup>5</sup>Department of Gastroenterology, Hepatology and Infectious Diseases, University of Magdeburg, Magdeburg, Germany.

**Introduction:** Gastric carcinoma (GC) represents the forth most common cancer type worldwide. To find genetic risk factors priming for GC development, GWAS in Asian populations have been performed already with success. However, genetic GC studies in patients of European origin are scarce and so far have focused on small sets of candidate loci only.

**Materials and Methods:** To identify germline variants playing a role in the development of GC in patients of European descent, we genotyped a set of 27 SNPs in a Eastern European sample comprising 502 GC cases and 507 controls. As disease associated variants often reside in gene regulatory elements, we selected the SNPs based on the latest data release of the Genotype-Tissue Expression (GTEx) consortium, focusing on strong stomach specific expression quantitative trait loci (eQTLs).

**Results:** Three of the tested SNPs showed at least nominal significance for the development of GC ( $P < 0.05$ ). SNP rs2976397 showed the strongest GC association signal ( $P = 2 \times 10^{-8}$ ) at the same time influencing the expression of *PSCA*. SNP rs198408 showed GC association ( $P = 1.67 \times 10^{-2}$ ) and regulates the expression of *NPPA*. Additionally, SNP rs10771539 was GC associated ( $P = 2.31 \times 10^{-2}$ ) regulating the transcription of *KLRB1*.

**Conclusion:** In summary, using stomach specific eQTLs we identified three SNPs that are associated with GC development. Further replication studies in a large independent European sample are currently performed to further elucidate the role of eQTLs in the development of this fatal disease.

**P12.098****Deciphering the genetic bases of gastric carcinoids: from human to mouse model.. A knockin mouse model with a missense mutation in the ATP4a gene mimics an aggressive familial form of gastric neuroendocrine tumor type I in humans**

*O. Calvete<sup>1</sup>, A. Varro<sup>2</sup>, P. Vargiu<sup>3</sup>, S. Dodd<sup>2</sup>, D. Pritchard<sup>2</sup>, M. Garcia<sup>4</sup>, J. Reyes<sup>5</sup>, S. Ortega<sup>3</sup>, J. Benítez<sup>1</sup>*

<sup>1</sup>Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain, <sup>2</sup>Department of cellular and molecular physiology. Institute of translational medicine. University of Liverpool, Liverpool, United Kingdom, <sup>3</sup>Transgenic mice core unit. Spanish national cancer research center (CNIO), Madrid, Spain, <sup>4</sup>Animal facility core unit. Spanish national cancer research center (CNIO), Madrid, Spain, <sup>5</sup>Department of gastroenterology. Hospital INCA, Majorca, Spain.

**Introduction:** The H,K-ATP4a proton pump acidifies the gastric lumen in tight coordination with gastrin. We recently identified a missense mutation in the human ATP4a gene that causes an aggressive form of familial gastric neuroendocrine tumor characterized by early age of onset, hypoacidity, hypergastrinemia, iron-deficiency anemia, muscular infiltration, intestinal metaplasia and adenocarcinoma. Total gastrectomy was undertaken as the final treatment for the affected patients.

**Materials and Methods:** We constructed a knockin mouse model for this mutation to better understand the pathology developing and search for new therapies

**Results:** Mice mimic most of the alterations described in human patients, including achlorhydria, iron-deficiency anemia and hypergastrinemia, which validate the mutation as the primary responsible for this disease. While mice developed severe hyperplasia, dysplasia and glandular metaplasia in the stomach, gastric neuroendocrine tumors or adenocarcinomas as observed in humans were not observed. Interestingly, gastric acidification by treatment with 3% HCl in drinking water, both prevented (treatment since birth) and reverted (treatment starting at 150 or 250 days after birth) the glandular metaplasia and severe dysplasia phenotype of mice and partially restored the biochemical parameters in stomachs.

**Conclusions:** Preliminary preclinical experiments with HCl drinking water prevent and revert the gastric pathology in mouse model. These results suggest a therapeutic alternative for the treatment of human patients. Furthermore, the knockin mouse model represents a unique and novel tool for studying pathologies related to stomach acid disturbances.

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**P12.099****Genomic classification of oral squamous cell carcinoma: the role of copy number alterations**

*I. P. Ribeiro<sup>1,2</sup>, F. Caramelo<sup>3</sup>, F. Marques<sup>2,4,5</sup>, A. Santos<sup>1</sup>, L. Barroso<sup>6</sup>, M. Mesquita<sup>6</sup>, T. Lopes<sup>6</sup>, M. J. Julião<sup>7</sup>, A. Ferreira<sup>8</sup>, I. P. Baptista<sup>2,4</sup>, J. B. Melo<sup>1,2</sup>, I. M. Carreira<sup>1,2</sup>*

<sup>1</sup>Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>2</sup>CIMAGO - Center of Investigation on Environment, Genetics and Oncobiology - Faculty of Medicine, University of Coimbra, Coimbra, Portugal,

<sup>3</sup>Laboratory of Biostatistics and Medical Informatics, IBILI - Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>4</sup>Department of Dentistry, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>5</sup>Stomatology Unit, Coimbra Hospital and University Centre, CHUC, EPE, Coimbra, Portugal, <sup>6</sup>Maxillofacial Surgery Unit, Coimbra Hospital and University Centre, CHUC, EPE, Coimbra, Portugal, <sup>7</sup>Department of Pathology, Coimbra Hospital and University Centre, CHUC, EPE, Coimbra, Portugal.

**Introduction:** Oral Squamous Cell Carcinoma (OSCC) is a phenotypic, aetiological, biological and clinical heterogeneous malignancy. The current attempts of risk stratification do not have utility in clinical routine and consequently in the patients survival. We aimed to molecularly discriminate these heterogeneous OSCC patients based in chromosomal gains and losses and consequently find a clinical meaning to the molecular groups identified. **Material and Methods:** Array-comparative genomic hybridization in 93 OSCC samples was performed using an Agilent oligonucleotide microarray 4x180K. Healthy donors were used as controls. **Results:** Our results confirmed the regions previously reported as altered in these tumors, including gain of chromosomes 3q, 8q and 11q13 and loss in 3p, 5q and 9p. The presence or absence of metastasis/relapses is one of the most important predictors of disease outcome. Using cluster analysis based only in the genomic profile we were able to separate our cohort in two groups, one of which was correlated with a high risk of metastasis/relapses development during or after treatment, ( $\chi^2(1)=6.7610637$ ;  $p=0.0093168$  and  $OR=3.868, IC95\% (1.478; 10.12)$ ). This specific genomic signature identified seems to have the power to predict outcome in OSCC with huge implications in terms of treatment choices, disease control and prevention. **Conclusions:** These results represent a significant step forward in the translation of genomic biomarkers for OSCC management in the clinical daily use.

**P12.100****genetic investigation of familial hematological malignancies**

*W. Hamadou<sup>1</sup>, S. Besbes<sup>1</sup>, R. El abed<sup>1</sup>, V. Bourdon<sup>2</sup>, Y. Ben youssef<sup>3</sup>, M. Laatiri<sup>4</sup>, S. Jemni<sup>5</sup>, T. Noguchi<sup>6</sup>, A. Khelif<sup>6</sup>, H. Sobol<sup>6</sup>, Z. Soua<sup>1</sup>*

<sup>1</sup>Faculty of Medicine of Sousse, Sousse, Tunisia, <sup>2</sup>Institut Paoli-Calmette, marseille, France, <sup>3</sup>Service of Clinical Hematology. CHU Farhat Hached, Sousse, Tunisia, <sup>4</sup>Service of Clinical Hematology. CHU Fattouma Bourguiba, monastir, Tunisia, <sup>5</sup>Regional center of Blood transfusion . CHU F. Hached, Sousse, Tunisia, <sup>6</sup>Institut Paoli-Calmette, Marseille, France.

**Introduction:** Familial aggregation among patients with several hematological malignancies has been revealed. This emphasizes the importance of genetic factors. Only few genes predisposing to familial hematological malignancies have been reported until now due to the low occurrence. Few variants that might contributing to the background of genetic factors were reported, this fact encourage us to extend our investigations to other co-operating genes. We target several genes previously reported in sporadic hematological malignancies as apoptotic genes (*FAS*, *FASLG*, *CASP8*, *CASP10*, *PRF1*), proto-oncogenes (*CRAF*, *BRAF*, *CBL*), tumor suppressor genes (*CEB-PA*, *TP53*, *RUNX1*) and genes involved in several cell process (*YY1*, *ASXL1*, *NPM1*, *JAK2*). **Materials and Methods:** In this study, we investigated these 15 candidate genes by direct sequencing in 20 Tunisian familial cases with aggregated hematological malignancies. **Results:** We report a new *ASXL1* germline missense substitution p.Arg402Gln in two related patients that we describe it for the first time in non-Hodgkin lymphoma. We report also a *PRF1* p.Ala211Val variant in two related patients both diagnosed with Hodgkin lymphoma. *In silico* analysis has predicted potential deleterious effect of these two variants and were totally absent in 200 control chromosomes. We report also two intronic substitutions: c.1641+6 T>C in *JAK2* gene and 559+1 G>A in *TP53* gene in familial cases diagnosed respectively with Hodgkin lymphoma and acute lymphoblastic leukemia. This two substitutions may disrupt the splice site and impact the normal protein function. **Conclusion:** From an extended candidate genes analyzed in the field of familial hematological malignancies, *ASXL1*, *TP53*, *JAK2* and *PRF1* might be involved. These variants should be considered as potential candidate genes since a potential damaging effect was predicted.

**P12.101****Establishment and characterization of a primary glioblastoma multiforme human cell line with C176F TP53 mutation**

*L. Muñoz-Hidalgo<sup>1</sup>, C. López-Ginés<sup>2</sup>, R. Gil-Benso<sup>2</sup>, S. Rodríguez<sup>3</sup>, J. Mejías<sup>2</sup>, T. San Miguel<sup>2</sup>, M. Cerdá-Nicolás<sup>2</sup>*

<sup>1</sup>Foundation for research HCU-INCLIVA, Valencia, Spain, <sup>2</sup>University of Valencia, Valencia, Spain, <sup>3</sup>Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

**Introduction:** Primary glioblastoma (GBM) is the most frequent and most malignant neoplasm of the human brain tumors which, despite current therapies, usually causes the patient's death within one year following diagnosis. Cell lines derived from primary tumors represent an important tool for studying the biological behavior of these malignant neoplasms and searching for new drugs and treatments. The objective of the present work was the immunohistochemical and genetic characterization of a new human cell line established from a primary GBM, named GBM-HC-44.

**Material and Methods:** We have analyzed immunohistochemical markers, cytogenetics and molecular characteristics of the cell line and the primary tumor. We have analyzed the variation in the number of copies and the methylation status of genes involved in cancer by MLPA technique (MLPA P105-D1 gliomas and ME001-C2 tumor suppressor-1). EGFR /CEP7 expression was analyzed by FISH technique. Likewise, sequencing for TP53, SKY and CGH array were performed.

**Comments:** We report the characterization of GBM-HC-44 cell line, derived from a primary GBM with C176F mutation in TP53 and without EGFR amplification. Numerical and structural alterations in chromosomes as trisomies 7, 14, 15, 18, 20 and 21, and further deletions in chromosomes regions 10, 13, 16 and 19 were detected. The study of GBM-HC-44 cell line and its comparison with features of the primary tumor may improve the knowledge about these neoplasms and could be a very useful material for basic research of GBM.

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## P12.102

### Early onset of Glioblastoma in Indian consanguineous family is caused by R802X PMS2 homozygous mutation

S. E. Antonarakis<sup>1</sup>, G. Chetan<sup>2</sup>, M. Adrianova<sup>3</sup>, V. Zoete<sup>4</sup>, M. Sibin<sup>2</sup>, K. Narasinga Rao<sup>5</sup>, O. Michielin<sup>6</sup>, P. Ribaux<sup>1</sup>, V. Seplyarskiy<sup>3</sup>, S. I. Nikolaev<sup>7</sup>;

<sup>1</sup>University of Geneva, Geneva, Switzerland, <sup>2</sup>Department of human genetics. Nimhans, Bangalore, India, <sup>3</sup>Institute of Information Transmission Problems, Russian Academy of Sciences, Moscow, Russian Federation, <sup>4</sup>SIB, Lausanne, Switzerland, <sup>5</sup>Department of Neurosurgery. Nimhans, Bangalore, India, <sup>6</sup>SIB, CHUV, Lausanne, Switzerland, <sup>7</sup>University Hospitals of Geneva, Geneva, Switzerland.

We have performed exome sequencing of two second cousin patients with the early onset of glioblastoma and their parents from a consanguineous family with likely autosomal recessive inheritance. 1.3% of the patients genomes were identical by descent and homozygous; the homozygous segment included a germline p.R802X variant in the PMS2 gene. We have confirmed that the mutation segregated with the phenotype. Additionally, we have performed the exome sequencing of the tumors, and revealed extremely high somatic mutation rates: one tumor contained 12621 somatic mutations including truncating in PARP1, ARID2, CREBBP and TP53; and the other - 6764 mutations including two drivers in PIK3CA (p.R88Q and p.T1025A) and truncating mutations in APC and in NF1. Interestingly both tumors harbored somatic mutations in the proofreading domain of POLE (p.P436H and p.L424V), polymerase that replicates the leading strand during DNA replication. In-silico modeling suggested these mutations cause significant structural instability of the protein. Most interestingly we have observed in both cancers that the vast majority of mutations were consistent with the signature of POLE exo-: C>A and C>T mutations which occurred on the leading strand. This genetic analysis revealed the cause and functional mechanism of familial glioblastoma and provided additional diagnostic opportunity in the studied family.

## P12.103

### Over expression of hMSH2 mRNA is associated with poor prognosis in high grade glioma

C. K. Ghati;

National Institute of Mental Health and Neurosciences, Bangalore, India.

Introduction: Glioblastoma and anaplastic oligodendrogloma are high grade gliomas (HGG). Human MutS homolog2 (*hMSH2*) is the most consistently expressed MMR gene and a key component for mismatch recognition. Proficient expression of *hMSH2* mRNA is important for efficient functioning of MMR, thereby maintaining micro-satellite and genomic stability. We studied the expression levels of *hMSH2* and its role in progression free survival (PFS) in high grade glioma.

Materials and Methods: Our study group consisted of 39 glioma patients (frontal lobe) and 8 non-glioma patients. Expression of *hMSH2* mRNA was studied using qRT-PCR (ABI Taqman Gene expression assay). Kruskal Wallis test was used for comparison of mRNA expression between various groups. Kaplan Meier survival analysis was used for variables with prognosis and Cox proportional hazard regression was used for multivariate analysis.

Results: Over expression of *hMSH2* with median fold change of 2.48 (Range-0.01-114.98) was found in 51.5% cases significantly different from non-glioma tissues ( $p=0.036$ ). Positive correlation was seen between *hMSH2* gene expression and age [ $r = +0.4$  ( $p=0.01$ )]. Age, histological type, grade, extent of resection and post surgery adjuvant therapy (RT+CT) were associated with PFS of patients in univariate analysis ( $p<0.05$ ). Low *hMSH2* expression with RT+CT was seen to have significantly better prognosis (median=22months), when compared to high expression with RT+CT (median=10months,  $p=0.011$ ). Tumor type, grade and effective therapy independently predicted PFS ( $p<0.05$ ) using multivariate analysis.

Conclusion: Over expression of *hMSH2* mRNA suggests higher number of mispair DNA mutations in high grade glioma. This can be used as a potential prognostic biomarker.

## P12.104

### Methylated MGMT alleles are distributed heterogeneously in glioma, irrespective of IDH status and chromosome 10q deletion

C. Pesenti<sup>1,2</sup>, L. Fontana<sup>2</sup>, S. Tabano<sup>2</sup>, E. Bonaparte<sup>1,2</sup>, R. Falcone<sup>1,2</sup>, G. Marfia<sup>2,3</sup>, M. Caroli<sup>1</sup>, S. Guerrieri<sup>1</sup>, M. Baccarin<sup>1</sup>, N. Carlessi<sup>1</sup>, S. Sirchia<sup>2</sup>, S. Bosari<sup>1,2</sup>, M. Miozzo<sup>1,2</sup>;

<sup>1</sup>Fondazione IRCCS Cà Granda-Ospedale Policlinico di Milano, Milano, Italy, <sup>2</sup>Università degli Studi di Milano, Milano, Italy, <sup>3</sup>Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico di Milano, Milano, Italy.

INTRODUCTION: The evaluation of molecular markers in glioma is part of clinical assessment and glioma classification.

Mutations in the dehydrogenases IDH1-IDH2 are prognostic markers in glioma, strongly associated with the epigenetic silencing of the O6-me-

thylguanine-DNA-methyltransferase (MGMT). Little is known about the timing of MGMT silencing during gliomagenesis, the distribution of MGMT methylated alleles and the potential association with MGMT deletion (10q LOH). We investigated the associations among MGMT methylation levels, IDH genotyping and 10q allelic loss.

METHODS: We quantitatively assessed by pyrosequencing the IDH1 R132H allele frequency and the MGMT methylation percentages in 208 primitive glioma samples. The 10q LOH was determined by Short Tandem Repeats genotyping or array-CGH.

RESULTS: 1. IDH1 was heterozygously mutated in virtually all cancer cells, whereas MGMT methylated alleles were not homogeneously distributed in tumor cells.

2. The MGMT methylation percentages directly correlated with increasing OS.
3. The MGMT LOH acts as a late event during gliomagenesis, not related to methylation.

CONCLUSION: The incomplete overlap between the percentages of IDH1 mutated and MGMT methylated alleles in tumor cells suggests that only a fraction of tumor cells undergo MGMT silencing, indicating an incomplete association between the two events. Nevertheless, MGMT methylation is a positive prognostic marker irrespective of IDH1 status. Finally, MGMT can be considered a tumor suppressor completely inactivated by 10q LOH and methylation in more advanced tumors.

## P12.105

### Identification of GPRC5A as a novel risk factor for the triple negative tumor phenotype in BRCA1 and BRCA2 mutation carriers

K. Klaschik<sup>1,2,3</sup>, E. Pohl<sup>1,2,3</sup>, J. Hauke<sup>1,2,3</sup>, G. Neidhardt<sup>1,2,3</sup>, S. Kröber<sup>1,2,3</sup>, S. Heilmann-Heimbach<sup>4,5</sup>, H. Hellebrand<sup>6</sup>, H. Surowy<sup>7</sup>, J. Altmüller<sup>8,9</sup>, P. Nürnberg<sup>8,9,10</sup>, C. Engel<sup>11</sup>, B. Wappenschmidt<sup>1,2,3</sup>, K. Rhiem<sup>1,2,3</sup>, A. Meindl<sup>8</sup>, R. Schmutzler<sup>1,2,3</sup>, E. Hahnen<sup>1,2,3</sup>;

<sup>1</sup>Center for Familial Breast and Ovarian Cancer, Cologne, Germany, <sup>2</sup>Center for Integrated Oncology (CIO), Cologne, Germany, <sup>3</sup>Center for Molecular Medicine Cologne (CMMC), Cologne, Germany, <sup>4</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>5</sup>Life&Brain Center, Bonn, Germany, <sup>6</sup>Department of Gynaecology and Obstetrics, Klinikum rechts der Isar at the Technical University, Munich, Germany, <sup>7</sup>Molecular Epidemiology, German Cancer Research Center (DKFZ) and Molecular Biology of Breast Cancer, Heidelberg, Germany, <sup>8</sup>Cologne Center for Genomics, Cologne, Germany, <sup>9</sup>Institute of Human Genetics, University of Cologne, Cologne, Germany, <sup>10</sup>Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany, <sup>11</sup>Institute for Medical Informatics, Statistics and Epidemiology (IMISE)Center for Familial Breast and Ovarian Cancer, Leipzig, Germany.

*GPRC5A* encodes an orphan G-protein coupled receptor highly expressed in lung tissue. *Gprc5a* knockout mice spontaneously develop lung adenomas and adenocarcinomas suggestive of a role in cancer predisposition. Within a consanguineous breast cancer (BC) family from Turkey, we identified a homozygous frameshift mutation (c.183delG; p.R61Sfs\*59) in the first coding exon of *GPRC5A* in two affected sisters via exome sequencing, suggesting an autosomal recessive trait. Interestingly, Sokolenko et al. (2014) reported a ten-fold increase of the heterozygous *GPRC5A* p.R61Sfs\*59 mutation in *BRCA1* c.5266dupC BC patients compared to *BRCA1*-negative BC patients. Thus, we hypothesized that *GPRC5A* acts as recessive BC gene and disease modifier when heterozygously inactivated. While the search for homozygous or compound heterozygous *GPRC5A* mutation carriers is ongoing, we genotyped the p.R61Sfs\*59 mutation in a large series of *BRCA1/2* mutation carriers ( $n=1,841$ ), *BRCA1/2*-negative familial cases ( $n=5,841$ ) and control individuals ( $n=8,305$ ). The detailed analysis of the *BRCA1/2* mutation carriers, consisting of 1,282 BC, 131 ovarian cancer (OC), 106 BC/OC patients and 322 healthy mutation carriers, revealed that the 17 *GPRC5A* mutation carriers solely coincide with the BC subgroup ( $CF=1,33\%$ ). Remarkably, the mutation was particularly frequent in *BRCA1/2*-positive triple negative BC cases (11/540,  $CF=2.04\%$ ) and presented a statistically significant association with this BC subtype (odds ratio: 2.362; 95%CI=1.177-4.627;  $p=0.007$ ). In summary, we provide evidence for *GPRC5A* p.R61Sfs\*59 as a disease modifier in *BRCA1/2* mutation carriers, especially in BC cases affected by the triple negative subtype. We are now starting functional analyses to elucidate the molecular mechanisms of *GPRC5A* p.Arg61fs in BC pathogenesis.

## P12.106

## Prostate cancer susceptibility assessed by GWAS risk variants in Finnish men

*C. Sipeky<sup>1</sup>, T. L. J. Tammela<sup>2</sup>, A. Auvinen<sup>3</sup>, t. PRACTICAL consortium<sup>4</sup>, J. Schleutker<sup>1,5</sup>;*  
<sup>1</sup>Department of Medical Biochemistry and Genetics, University of Turku, Turku, Finland, <sup>2</sup>Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland, <sup>3</sup>Department of Epidemiology, School of Health Sciences, University of Tampere, Tampere, Finland, <sup>4</sup>Members from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium are provided in the Supplement/foot notes., <sup>5</sup>Tyks Microbiology and Genetics, Department of Medical Genetics, Turku University Hospital, Turku, Finland.

**Introduction:** Prostate cancer (PrCa) is the most common cancer in males in Finland with 5043 new cases in 2015. Genome-wide association studies (GWAS) identified 101 common PrCa susceptibility loci, explaining 33% of familial risk of prostate cancer predominantly in mixed European population. Our aim is to assess the association of selected GWAS "hot hits" with PrCa specifically in Finnish men.

**Materials and methods:** In order to subset GWAS variants with strong risk effect we applied the per allele odds ratio (OR) of  $\geq 1.1$ , and with strong protective effect the OR  $\leq 0.9$ . Effect allele frequency was chosen as  $> 0.1$ . By applying these criteria we selected 37 known risk and 13 known protective variants from 101 GWAS hits in PrCa. Germline DNA samples for common PrCa risk loci were genotyped for 5165 Finnish subjects (n=2764 unselected cases, n=2401 controls) within the Collaborative Oncological Gene-Environment Study (COGS) using Illumina iSelect custom SNP genotyping platform. Statistical analyses of case-control logistic regression model was performed by using PLINK software.

**Results and Conclusions:** 13 of 37 common European risk variants (1/3), and 4 of 13 common European protective variants were not found as statistically significant on GWAS level in Finnish samples. The highest PrCa susceptibility variant is at 8q24 CASC8 gene region ( $p=5.26 \times 10^{-12}$ ), which seems to play an important role in PrCa genetics in Finns.

\* Information of the consortium can be found at <http://practical.ccge.medschl.cam.ac.uk/>.

## P12.107

## Post-mortem testing: Germline variant detection in genes related to hereditary breast, ovarian and colorectal cancer using archival non-tumor tissue

*T. D. Jensen, A. Petersen, M. Joergensen, A. Bojesen;*  
*Department of Clinical Genetics, Lillebaelt Hospital, Vejle, Denmark.*

**Introduction:** A sufficient evaluation of cancer risk in a family suspected of e.g. hereditary breast and ovarian cancer (HBOC) or Lynch syndrome very often requires genetic testing. The task of identifying the disease-causing mutation in a family is difficult without affected living family members. Identifying a mutation in tissue-derived DNA from deceased is technically challenging and because of that, this diagnostic approach has not been offered on a regular basis by diagnostic departments or companies.

**Materials and Methods:** DNA was extracted from 9x15  $\mu\text{m}$  FFPE tissue sections per sample, and DNA integrity was estimated. DNA samples were subjected to HaloPlex Target Enrichment using a custom design (Agilent Technologies). After enrichment, HaloPlex libraries were diluted, pooled, denatured and 10 pM library pool was subjected to paired-end (2x150 bp), single index (8 bp) DNA sequencing on a MiSeq (Illumina).

**Results:** In clinical use, a total of 304 samples (0-43 years) were investigated using our HBOC and colorectal cancer NGS panels. We identified 16 pathogenic and 1 likely pathogenic BRCA1 variants, 11 pathogenic and 1 likely pathogenic BRCA2 variants, 7 pathogenic and 1 likely pathogenic CDH1 variants and 1 pathogenic PALB2 variant. Furthermore, we identified 5 pathogenic APC variants. We did not detect any pathogenic variants in the Lynch syndrome-associated genes MLH1, MSH2, MSH6, PMS2 and EPCAM.

**Conclusions:** The possibility of identifying pathogenic mutations in deceased family members is a new and important tool in the process of determining or evaluating the risk of certain cancers of family members who receive genetic counseling.

## P12.108

## Famosa study: evaluation of a multigene panel in patients with suspected HBOC

*E. Adrover<sup>1</sup>, I. Esteban<sup>2</sup>, A. G. Calabuig<sup>1</sup>, G. Llort<sup>3</sup>, S. Servitja<sup>4</sup>, A. Nuñez<sup>5</sup>, I. Garau<sup>6</sup>, J. M. Cano<sup>7</sup>, R. Serrano<sup>8</sup>, M. López<sup>9</sup>, S. González<sup>9</sup>, M. Juan<sup>10</sup>, C. Alonso<sup>11</sup>, A. Casas<sup>12</sup>, B. Graña<sup>13</sup>, A. Teulé<sup>14</sup>, A. Marquez<sup>15</sup>, J. Alés<sup>16</sup>, C. Guillén<sup>17</sup>, A. Antón<sup>18</sup>, J. Brunet<sup>19</sup>, F. Balaguer<sup>20</sup>, J. Balmañá<sup>2</sup>,*

<sup>1</sup>C.H. Universitario Albacete, Albacete, Spain, <sup>2</sup>H Vall Hebrón, Barcelona, Spain, <sup>3</sup>H. Parc Taulí, Sabadell, Spain, <sup>4</sup>H del Mar, Barcelona, Spain, <sup>5</sup>H Mataró, Mataró, Spain, <sup>6</sup>H. Son Llatzer, Palma Mallorca, Spain, <sup>7</sup>HG Ciudad Real, Ciudad Real, Spain, <sup>8</sup>H. Reina Sofia, Córdoba, Spain, <sup>9</sup>H. S. Pedro Alcántara, Cáceres, Spain, <sup>10</sup>IVO, Valencia, Spain, <sup>11</sup>H. de la Princesa, Madrid, Spain, <sup>12</sup>H. Virgen Rocío, Sevilla, Spain, <sup>13</sup>H. Abente y Lago, Coruña, Spain, <sup>14</sup>ICO Hospital, Hospitalet, Spain, <sup>15</sup>H. Clínico Málaga, Málaga, Spain, <sup>16</sup>H. Ntra. Sra. Soñsoles, Avila, Spain, <sup>17</sup>H. Ramón y Cajal, Madrid, Spain, <sup>18</sup>H. Miguel Servet, Zaragoza, Spain, <sup>19</sup>H Josep Trueta, Gerona, Spain, <sup>20</sup>H. Clínico, Barcelona, Spain.

**OBJECTIVES:** Characterize the frequency of mutations in patients with clinical criteria for Hereditary Breast and Ovarian Cancer (HBOC) using a 25-gene panel in a Spanish population (FAMOSA study).

**PATIENTS:** Patients with breast or ovarian cancer who met the NCCN criteria for genetic testing with a) prior testing for BRCA genes with NO mutation identified; or b) recently diagnosed (<6 months) and not genetically tested, were enrolled for multiplex cancer testing.

**RESULTS:** From November 14 to February 15, 210 patients were included in the FAMOSA study (109 HBOC). 61 (56%) patients were previously tested for BRCA1/2 gene mutations with conventional techniques; median age: 44y (22-77); gender: 3 males / 106 females; cancer types: breast 96 (87.3%); ovary 14 (12.7%). Overall 23 (21%) pathogenic variants were identified in 19 patients; 10 BRCA1, 2 BRCA2, 2 PALB2, 3 MYH, 1 CDKN2A; 3 ATM, 1 BRAD1, 1 BRIP1. One patient had an unexpected mutation in CDKN2A gene (gluteus sarcoma age 20; bilateral breast ca; ages 45 and 50; father lung ca, age 70; brother melanoma, age 35). Of 61 patients previously tested, 1 had a pathogenic variant in BRCA1 and 17/19 patients with VUS were classified negative in BRCA genes with the 25-gene panel.

**CONCLUSIONS:** Panel testing in patients with HBOC yielded a 21% mutation rate, increasing the yield of genetic mutations beyond BRCA; one unexpected finding in CDKN2A was identified.

## P12.109

## Identification of novel candidate genes for Hereditary Breast Cancer via Whole Exome Sequencing

*D. Turchetti<sup>1,2</sup>, R. Zuntini<sup>1,2</sup>, F. Isidori<sup>1</sup>, C. Diquigiovanni<sup>1</sup>, F. Buscherini<sup>1</sup>, F. Palombo<sup>1</sup>, S. Miccoli<sup>1,2</sup>, E. Bonora<sup>1,2</sup>;*

<sup>1</sup>U.O.Genetica Medica, Policlinico S.Orsola-Malpighi, Bologna, Italy, <sup>2</sup>Centro di Ricerca sui Tumori Ereditari, Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, Bologna, Italy.

Hereditary breast cancer (HBC) accounts for 5-10% of breast cancer cases. Germline BRCA1/2 mutations explain 60% of HBC, but no other major genes have been identified in the remaining 40%.

We decided to perform whole exome sequencing (WES) in affected first-degree cousin-pairs displaying features suggestive of HBC, who tested negative for BRCA1/2. Based on their relationship, cousins share 1/8 of the entire genome, which helps filter and select candidate variants. To date, 4 cousin-pairs were analyzed through WES and candidate variants were identified in three different genes in 3 pairs, whereas the fourth one shared a BRCA2 deletion not previously identified by Sanger sequencing.

The candidate variants, affecting *ROS1*, *RASAL1* and *ICAM5* genes, were confirmed by direct sequencing. Further characterization of the variants is ongoing. In particular, the *ROS1* variant affected a predicted canonical splice site, suggesting an effect on the correct splicing of *ROS1*, a tyrosine kinase receptor that has been implicated in several cancer types. We inserted the wild-type or mutant genomic region into a minigene plasmid, transfected it into COS7 cells, and found that the mutant cells used a cryptic intronic splice site 20 bp downstream, leading to the insertion of a premature stop-codon in the protein. Future studies will explore the effect of the variant on cell growth/proliferation and invasion in CRISP/Cre-modified MCF10A and MCF7 cells.

Our findings support previous evidence that many different genes account for the fraction of HBC not related to BRCA1/2 mutations and led us to identify promising novel variants for HBC.

## P12.110

### Search for new high susceptibility genes in hereditary breast cancer families with a recessive pattern of inheritance

A. Távera<sup>1</sup>, M. de la Hoya<sup>2</sup>, A. Macías<sup>3</sup>, A. Barroso<sup>1</sup>, M. Urioste<sup>1</sup>, J. Benítez<sup>1</sup>, A. Osorio<sup>1</sup>;  
<sup>1</sup>CNIO, Madrid, Spain, <sup>2</sup>Hospital Clínico San Carlos, Madrid, Spain, <sup>3</sup>Hospital Morales Meseguer, Murcia, Spain.

Only two high-risk genes involved in hereditary breast and ovarian cancer (HBOC) have been identified, BRCA1 and BRCA2, that do not explain more than 20% of HBOC cases. In the last years, Whole Exome Sequencing (WES), has been used to find novel susceptibility genes.

So far, dominant or polygenic patterns of inheritance have been considered, however, some publications have implemented simulations with different models of segregation in a large number of families which suggests that a part of them could be explained by a model of recessive inheritance, a hypothesis that has remained unexplored.

Five families showing an apparent pattern of recessive inheritance were selected based on: presence of two or more affected siblings with BC at young age, absence of familial antecedents of the disease, availability of the samples and no mutations in BRCA1/BRCA2 genes. Through WES, 23 candidate variants following an apparent recessive inheritance model were identified and validated by Sanger sequencing. Segregation and loss of heterozygosity analyses in tumors samples are being performed. A further analysis considering a dominant inheritance pattern was also performed. 30 candidate variants were found in this analysis, including a truncating mutation in ATM, a known HBOC moderate risk gene, which lead us to the conclusion that we cannot rule out other models of inheritance. Case-control association study in Spanish population is being performed to get insights about the transcendence of these variants in the disease.

Grant References: La-Caixa fellowship, CNAG as part of the project "300 exomes to elucidate rare diseases" (2013)

## P12.111

### Introducing multigene panel testing for hereditary breast and ovarian cancer into routine practice: frequency of detected mutations in Slovene patients

A. Blatnik, M. Krajc, V. Stegel, P. Cerkovnik, S. Novaković;  
Institute of Oncology, Ljubljana, Slovenia.

Introduction: Next generation sequencing (NGS) of breast/ovarian cancer susceptibility genes has recently been shown to be an accurate and cost-effective method of performing genetic testing. NGS offers the possibility of screening patients for mutations in high and moderate risk genes other than BRCA1/2. Subsequently, it increases the detection rate of variants of uncertain significance. Our study aimed to determine the frequency of germline mutations found in Slovene patients using an NGS multigene panel.

Methods: We retrospectively collected and analyzed data from a group of 306 patients selected in accordance with standard BRCA1/2 testing guidelines and sequenced at our institution between October 2014 and November 2015. We assessed the frequency of mutations in genes associated with a predisposition to breast/ovarian cancer, such as BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, CHEK2, PALB2 and ATM.

Results: Pathogenic variants were detected in 29.4% of our patients. Most mutations were identified in BRCA1 (16.7%) and BRCA2 (7.8%). In addition, 5.2% carried a mutation in another breast/ovarian cancer susceptibility gene: 1.9% in CHEK2, ~1% in PALB2, ~1% in ATM, ~1% in CDH1 and 0.3% in TP53. One patient carried mutations in both BRCA2 and ATM.

Conclusions: With NGS panel testing we were able to detect pathogenic variants in genes other than BRCA1/2 in ~5% of our patients. CDH1 mutations were detected in patients with no family history of diffuse gastric cancer and a TP53 mutation was seen in a family with no Li-Fraumeni cancers apart from early-onset breast carcinoma, highlighting the benefits of this new approach.

## P12.112

### Molecular characterization of BRCA1/BRCA2 large genomic rearrangements in 1542 hereditary breast/ovarian cancer cases in Catalonia

S. Bonache<sup>1</sup>, A. Ruiz<sup>2</sup>, A. Tenés<sup>3</sup>, I. Hernan<sup>4</sup>, E. Carrasco<sup>5</sup>, N. Tuset<sup>6</sup>, I. Esteban<sup>5</sup>, N. Gadea<sup>7</sup>, G. Montalban<sup>1</sup>, C. Yagüe<sup>8</sup>, M. Segu<sup>9</sup>, A. Arcusa<sup>10</sup>, G. Llort<sup>8</sup>, J. Balmaña<sup>5,7</sup>, S. Gutiérrez-Enríquez<sup>1</sup>, O. Díez<sup>1,3</sup>;

<sup>1</sup>Oncogenetics Group, Vall d'Hebron Institute of Oncology-VHIO, Barcelona, Spain,

<sup>2</sup>Laboratori de Genètica, UDIAT-Centre Diagnòstic, Corporació Sanitària Parc Taulí, Institut Universitari Parc Taulí-UAB, Sabadell, Spain, <sup>3</sup>Area of Clinical and Molecular Genetics, Vall d'Hebron University Hospital, Barcelona, Spain, <sup>4</sup>Unitat de Genètica Molecular, Hospital de Terrassa (CST), Terrassa, Spain, <sup>5</sup>High Risk and Cancer Prevention Group, Vall d'Hebron Institute of Oncology-VHIO, Barcelona, Spain, <sup>6</sup>Medical Prevention Group, Vall d'Hebron Institute of Oncology-VHIO, Barcelona, Spain

<sup>7</sup>Oncology Department, Hospital Universitari Arnau de Vilanova, Lleida, Spain, <sup>8</sup>Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain, <sup>9</sup>Unitat de Consell Genètic. Corporació Sanitària Parc Taulí. Consorci Sanitari de Terrassa, Sabadell, Spain, <sup>10</sup>Departament d'Oncologia Mèdica. Corporació Sanitària Parc Taulí Institut Universitari-UAB, Sabadell, Spain, <sup>11</sup>Departament d'Oncologia Mèdica. Consorci Sanitari de Terrassa, Terrassa, Spain.

Pathogenic variants of the BRCA1 and BRCA2 genes (BRCA1/2) mostly include small insertions or deletions and nucleotide substitutions that generate a premature stop codon. Besides, large genomic rearrangements (LGRs) account for 5-10% of all deleterious variants in BRCA1/2.

Molecular characterization of LGRs is essential to know their genetic mechanisms, identify recurrent alterations, and investigate genotype/phenotype relationships.

We used multiplex ligation-dependent probe amplification (MLPA) or multiple amplicon quantification (MAQ) to identify BRCA1/2 LGRs in 1542 hereditary breast/ovarian cancer families referred to three hospitals in Catalonia.

We used long-range PCR, CGH arrays, and cDNA analyses to characterize the alterations at molecular level. We found 12 LGRs in BRCA1 and five in BRCA2.

Breakpoints were fully characterized in fourteen of them. Six LGRs were novel: deletions targeting exons 1-2, exon 8, exons 16-17, two different deletions of exon 24 in BRCA1 and one duplication of exon 21 in BRCA2. The BRCA1 deletion of exons 16-17 appears to be a Catalan founder mutation explaining five independent families.

Both non-homologous and homologous events were involved in the aetiology of the rearrangements. The cDNA analyses showed chimeric RNAs expressed by BRCA1 alleles carrying exons 1-2 or exon 24 deletions.

Our study substantially increases the spectrum of BRCA1/2 LGRs fully characterized at the molecular level. Also highlights the relevance of cDNA analysis for a reliable prediction of protein effect of LGRs involving 5' and 3'UTR expression regulatory regions.

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## P12.113

### Transcriptional analysis of 36 BRCA1/BRCA2 variants with potential effects on splicing

G. Montalban<sup>1,2</sup>, S. Bonache<sup>1</sup>, A. Tenés<sup>3</sup>, E. Carrasco<sup>4</sup>, N. Tuset<sup>5</sup>, I. Esteban<sup>4</sup>, N. Gadea<sup>6</sup>, J. Balmaña<sup>4,6</sup>, O. Díez<sup>1,3</sup>, S. Gutiérrez-Enríquez<sup>1</sup>;

<sup>1</sup>Oncogenetics Group, Vall d'Hebron Institute of Oncology-VHIO, Barcelona, Spain,

<sup>2</sup>Universitat de Barcelona, Barcelona, Spain, <sup>3</sup>Area of Clinical and Molecular Genetics, Vall d'Hebron University Hospital, Barcelona, Spain, <sup>4</sup>High Risk and Cancer Prevention Group, Vall d'Hebron Institute of Oncology-VHIO, Barcelona, Spain, <sup>5</sup>Medical Oncology Department, Hospital Universitari Arnau de Vilanova, Lleida, Spain, <sup>6</sup>Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain.

Introduction: Molecular diagnosis of BRCA1 and BRCA2 genes (BRCA1/2) often identifies variants of unknown significance (VUS). An evaluation of their effect on mRNA splicing becomes essential to elucidate their clinical impact. In this study we perform experimental and bioinformatic analysis to assess the role in disease development of a set of 36 VUS identified in Spanish high-risk breast/ovarian cancer families.

Methods: Bioinformatic tools were used to select 15 BRCA1 and 21 BRCA2 VUS with potential splicing effects from 1200 patients screened for BRCA1/2 pathogenic variants in genomic DNA. RNA from VUS carriers and 10 control samples was isolated from blood leukocytes. All variants are assessed by RT-PCR, agarose gels and direct sequencing. A semiquantitative capillary electrophoresis of fluorescent amplicons is performed to estimate the proportion of aberrant and normal transcripts. The allele-specific transcript expression will also be determined.

Results: Aberrant splicing patterns were observed in 5/15 BRCA1 and in 7/21 BRCA2 variants. A preliminary semiquantitative analysis of the BRCA1 c.4675+1G>C and BRCA2 c.8023A>G shows differences in the abundance of the Δ15 and Δ18 isoforms compared to control samples, respectively. In both cases, the full-length transcript exhibits a significant reduced expression compared to controls, supporting the hypothesis that the variant alleles only generate aberrant transcripts.

Conclusions: Our findings highlight semiquantitative capillary electrophoresis as an essential tool to allow the clinical classification of VUS.

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**P12.114****Genetic testing for hereditary cancer: is exome sequencing ready or there is still room for ad hoc designed panels?**

*M. Gausachs<sup>1</sup>, L. Feliubadaló<sup>1</sup>, R. Tonda<sup>2,3</sup>, J. Trotta<sup>2,3</sup>, E. Castellanos<sup>4</sup>, A. López-Doriga<sup>1</sup>, À. Teulé<sup>1</sup>, E. Tornero<sup>1</sup>, J. del Valle<sup>1</sup>, B. Gel<sup>4</sup>, M. Gut<sup>2,3</sup>, M. Pineda<sup>1</sup>, S. González<sup>1</sup>, M. Menéndez<sup>1</sup>, M. Navarro<sup>1</sup>, G. Capellá<sup>1</sup>, I. Gut<sup>2,3</sup>, E. Serra<sup>4</sup>, J. Brunet<sup>1</sup>, S. Beltran<sup>2,3</sup>, C. Lázaro<sup>1</sup>;*  
*<sup>1</sup>Hereditary Cancer Program, Joint Program on Hereditary Cancer Catalan Institute of Oncology, IDIBELL, Hospital de Llobregat, Spain, <sup>2</sup>Centro Nacional de Análisis Genómico (CNAG-CRG), Center for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>3</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>4</sup>Genetic Variation in Cancer Group, Joint Program on Hereditary Cancer, Institut de Medicina Predictiva i Personalitzada del Càncer, Badalona, Spain.*

**Introduction:** Several Next Generation Sequencing (NGS) panels have been developed for hereditary cancer diagnosis, although there is a debate about its cost-effectiveness compared to exome sequencing. The performance of two hereditary cancer panels is compared to exome sequencing.

**Materials and Methods:** Twenty-four patients were selected: ten with identified pathogenic mutations (control set) and 14 suspicious of hereditary cancer but without any identified mutation (discovery set). Two panels, TruSight-Cancer (94 genes) and a custom panel (122 genes), were assessed alongside exome sequencing.

**Results:** Eighty-three genes were targeted by all approaches. More than 99% of the bases had read depth over 30x (C30) in the panels whereas exome covered 94% of these positions at C30. Variant calling identified the 10 pathogenic mutations in the control set except for MSH6 mutation c.255dupC in TruSight-Cancer. Two hundred and forty unique non-silent coding and canonical splice-site variants were identified in the remaining 14 samples in the 132 panel genes, 7 of them putatively pathogenic in ATM, BARD1, CHEK2, ERCC3, FANCL, FANCM and MSH2. The three approaches identified a similar number of variants in the shared genes. In contrast, WES provides additional information. Exomes were on average three times more expensive than panel analyses.

**Conclusions:** Ad hoc panels are an option for genetic diagnostics for hereditary cancer. Benefits include lower cost and higher depth resulting in more positions covered sufficiently for clinical interpretation. However WES identifies variants in genes that are not currently targeted by panels.

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**P12.115****Identification of novel causal genes of hereditary colorectal cancer by performing whole-exome sequencing in individual high-risk families**

*N. Seguí<sup>1</sup>, F. Bellido<sup>1</sup>, M. Navarro<sup>1</sup>, L. Mina<sup>2</sup>, T. Pons<sup>3</sup>, M. Pineda<sup>4</sup>, R. Valdés-Mas<sup>4</sup>, N. Köger<sup>5</sup>, G. Aiza<sup>1</sup>, P. Mur<sup>4</sup>, R. Sanz-Pamplona<sup>6</sup>, S. Belhadj<sup>1</sup>, S. Iglesias<sup>1</sup>, J. Soto<sup>7</sup>, M. Urioste<sup>8</sup>, T. Caldés<sup>9</sup>, M. Balbín<sup>10</sup>, P. Blay<sup>11</sup>, D. Rueda<sup>12</sup>, M. Durán<sup>13</sup>, A. Valencia<sup>13</sup>, I. Blanco<sup>14</sup>, J. Brunet<sup>14</sup>, V. Moreno<sup>6</sup>, G. Plotz<sup>1</sup>, J. Surrallés<sup>2</sup>, X. S. Puente<sup>1</sup>, C. Lázaro<sup>1</sup>, G. Capellá<sup>1</sup>, L. Valle<sup>1</sup>;*  
*<sup>1</sup>Hereditary Cancer Program, Catalan Institute of Oncology, IDIBELL, Hospital de Llobregat, Barcelona, Spain, <sup>2</sup>Genome Instability and DNA Repair Group, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>3</sup>Structural Biology and Biocomputing Program, Spanish National Cancer Research Center (CNIO), Madrid, Spain, <sup>4</sup>Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Oviedo, Spain, <sup>5</sup>Biomedical Research Laboratory, Medizinische Klinik 1, Universitätsklinikum Frankfurt, Frankfurt, Germany, <sup>6</sup>Unit of Biomarkers and Susceptibility, Catalan Institute of Oncology, IDIBELL, Hospital de Llobregat, Barcelona, Spain, <sup>7</sup>Molecular Genetics Laboratory, Elche University Hospital, Elche, Spain, <sup>8</sup>Familial Cancer Clinical Unit, Human Cancer Genetics Program, Spanish National Cancer Centre (CNIO), Madrid, Spain, <sup>9</sup>Laboratorio de Oncología Molecular, Servicio de Oncología Médica, Hospital Clínico San Carlos, Madrid, Spain, <sup>10</sup>Laboratorio de Oncología Molecular, Instituto Universitario de Oncología del Principado de Asturias, Hospital Universitario Central de Asturias, Oviedo, Spain, <sup>11</sup>Familial Cancer Unit, Department of Medical Oncology, Instituto Universitario de Oncología del Principado de Asturias, Hospital Universitario Central de Asturias, Oviedo, Spain, <sup>12</sup>Laboratory of Molecular Biology, 12 de Octubre University Hospital, Madrid, Spain, <sup>13</sup>Instituto de Biología y Genética Molecular, IBGM-UVA-CSIC, Valladolid, Spain, <sup>14</sup>Hereditary Cancer Program, Catalan Institute of Oncology, IDIBGI, Girona, Spain.*

**Introduction:** Inherited factors account for over 20% of all colorectal cancers (CRC), but less than 6% can be explained by rare high-penetrance mutations in known genes. We aimed at identifying novel hereditary cancer genes by performing whole-exome sequencing in individual hereditary CRC families. **Materials and Methods:** Exome enrichment (Agilent SureSelect Human All Exon 50Mb) followed by massively parallel sequencing (Illumina Hi-Seq2000) was performed on blood DNA from at least 3 cancer-affected family members. Three Amsterdam-positive families were studied. Data analysis was performed using conventional algorithms. Validation studies in familial cancer series and in silico and in vitro functional studies were also carried out.

**Results:** One family harbored two mutations in the MUTYH gene -known polyposis (recessive) gene-, one recurrent in the European population and

the other novel, for which functional studies demonstrated its deleterious nature. The family showed an atypical phenotype for MUTYH, characterized by the absence of polyps, apparent autosomal dominant inheritance, and presence of a mismatch repair-deficient tumor.

The study of the second family allowed us to identify a novel hereditary CRC gene, FAN1. Functionally relevant mutations were identified in almost 3% of Amsterdam-positive families.

The third family carried of two mutated genes: a splice-site mutation in a tumor suppressor gene, and a missense mutation in a previously-proposed cancer-predisposing gene.

**Conclusions:** The analysis of exomes in individual high-risk families allowed us to identify two novel genes for hereditary CRC, as well as mutations in previously-known or -proposed CRC-predisposing genes.

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**P12.116****Increased utilization of multi-gene panel testing for hereditary cancer: overcoming initial barriers**

*E. K. Dalton, K. Fulk, A. F. Yussuf, R. McFarland, H. LaDuka, J. S. Dolinsky; Ambry Genetics, Aliso Viejo, CA, United States.*

Multi-gene panels (MGP) for hereditary cancer susceptibility became commercially available in the United States in 2012. Both genetics and non-genetics providers experienced initial concerns regarding utility and implementation of MGP. However, research has demonstrated multiple benefits of MGP, such as a higher diagnostic yield. This study analyzes the utilization of MGP over a three-year period at a clinical diagnostic laboratory in the U.S. Quarterly ordering trends for single-syndrome tests were compared to smaller and larger MGP from the third quarter (Q3) of 2013 to Q3 2015. Ordering provider (OP) specialties and genetics provider (geneticist, genetic counselor, advance practice nurse, or other clinician with genetics training) extent of involvement was also noted. Statistical analysis was performed using the Fisher's exact test.

Comparison of single-syndrome tests in Q3 2013 versus Q3 2015 revealed that larger MGP significantly increased in frequency from 39% to 65% ( $p<0.01$ ). MGP have been utilized more frequently across all OP specialties in 2015 compared to 2013. Genetics provider involvement significantly increased the utilization of MGP across all specialties ( $p<0.01$ ).

Consistent with recent literature, this study demonstrates markedly increased utilization of MGP compared to 2012 when initially available. However, despite the overall decrease, single-syndrome and smaller MGP tests still account for 34% of total tests in Q3 2015, demonstrating clinicians' desires for tiered testing options. Additional research is needed to directly investigate OP attitudes towards MGP, however this study indicates that initial concerns surrounding MGP have lessened over time, particularly when genetics providers are involved in the ordering process.

**P12.117****Technological innovation in hereditary cancer risk assessment**

*A. Kulkarni<sup>1</sup>, A. Kenney<sup>2</sup>, V. Tripathi<sup>1</sup>, C. Compton<sup>1</sup>, S. Rose<sup>1</sup>, E. Haque<sup>1</sup>, D. Ruddy<sup>1</sup>, L. Izatt<sup>1</sup>, A. Shaw<sup>1</sup>;*  
*<sup>1</sup>Guy's Hospital, London, United Kingdom, <sup>2</sup>UBQO Limited, London, United Kingdom.*

Guy's Cancer Genetics guidelines are used across Southeast London, Kent & East Sussex and updated in line with national standards. However they are currently presented in 'non-interactive' .pdf format that is not always readily accessible. It is also difficult to ensure clinicians are using the most recent version of the guidelines.

The Cancer Genetics application (App), developed by Guy's Clinical Genetics department and UBQO with funding from Guy's and St Thomas' Charity, provides streamlined hereditary cancer risk assessment and referral guidance for clinicians.

The aim is to address inequity in referrals and improve patient access to our service by targeting several key factors, including clinicians' limited understanding of hereditary cancer, lack of time, confusion about where to refer patients and use of outdated guidance.

The App contains a risk assessment tool and succinct reference guide, enabling clinicians to easily decide who requires genetic assessment of their cancer risk and who can be managed in primary or secondary care. Patients thereby have quicker access to cancer surveillance, genetic counselling, pharmacoprevention and surgical options.

Cancer Genetics is freely available on iOS and android platforms and via a public website. It is certified as a Class 1 medical device and a secure content management system allows quick central updates.

We will present the development process, user feedback and integration of

the App into primary and secondary care. Our goal is that Cancer Genetics will promote timely, evidence-based management of those at risk of hereditary cancer.

#### P12.118

#### Exome sequencing identified potential causative candidate genes for hyperplastic polyposis syndrome

*S. Peters<sup>1</sup>, I. Spier<sup>1</sup>, C. Trueck<sup>1</sup>, J. Altmueller<sup>2,3</sup>, K. Kayser<sup>1</sup>, M. Kloth<sup>4</sup>, S. Holzapfel<sup>1</sup>, R. Adam<sup>1</sup>, H. Thiele<sup>2</sup>, M. M. Noethen<sup>2,5</sup>, S. Aretz<sup>1</sup>;*

<sup>1</sup>Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Cologne Center for Genomics, University of Cologne, Cologne, Germany, <sup>3</sup>Institute of Human Genetics, University of Cologne, Cologne, Germany, <sup>4</sup>Institute of Pathology, University of Cologne, Cologne, Germany, <sup>5</sup>Department of Genomics, Life & Brain Center, Bonn, Germany.

Hyperplastic polyposis syndrome (HPS) is a poorly defined colorectal cancer predisposition characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. To date, only few molecular signatures have been described and neither the etiology of the syndrome nor the distinct genetic alterations have been identified.

To uncover predisposing causative genes, the exomes of 31 clinically well characterized HPS patients have been sequenced (Illumina HiSeq) using leukocyte DNA. The variants were filtered for rare (homozygous/compound heterozygous: MAF≤ 1%, heterozygous: 0.01% according to dbSNP, EVS, and ExAC), truncating, and missense germline mutations (pathogenic by ≥ 2/3 prediction tools). For data analysis and filtering the GATK software and the Cartagenia Bench Lab NGS Software were applied. In a first preliminary analysis, known cancer genes and candidate genes were analyzed.

After stringent filtering steps and manual inspection of the variants, potentially biallelic variants were found in 289 genes. However, none of the cancer genes contained a biallelic mutation that met the criteria. All in all, 943 genes harbored heterozygous mutations in at least two patients, eight of which are cancer genes (*ATM*, *CHEK2*, *ERCC4*, *JAK2*, *MET*, *NUP214*, *RNF43*, *USP6*). *ATM* and *RNF43* harbored recurrent mutations. These patients had variants in several cancer or candidate genes.

Preliminary data indicate that exome sequencing might identify relevant genes for HPS. However, the number of variants per patient is also in line with an oligogenic etiology of polyp predisposition. The current work-up includes the inclusion of non-cancer genes and validation of variants by Sanger sequencing.

#### P12.119

#### Multilocus Inherited Neoplasia Alleles Syndrome (MINAS) - A Case Series and Literature Review

*J. Whitworth<sup>1</sup>, A. Skytte<sup>2</sup>, L. Sunde<sup>2</sup>, D. Lim<sup>3</sup>, M. Arends<sup>4</sup>, L. Happerfield<sup>5</sup>, I. Frayling<sup>6</sup>, R. van Minkelen<sup>7</sup>, E. Woodward<sup>8</sup>, M. Tischkowitz<sup>1</sup>, E. Maher<sup>4</sup>;*

<sup>1</sup>University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>Aarhus University Hospital, Aarhus, Denmark, <sup>3</sup>Birmingham Women's Hospital, Birmingham, United Kingdom,

<sup>4</sup>University of Edinburgh and Institute for Genetics and Molecular Medicine, Edinburgh, United Kingdom, <sup>5</sup>Cambridge University Hospitals, Cambridge, United Kingdom, <sup>6</sup>Cardiff University School of Medicine, Cardiff, United Kingdom, <sup>7</sup>Erasmus Medical Center, Rotterdam, Netherlands, <sup>8</sup>Central Manchester University Hospitals, Manchester, United Kingdom.

Mendelian causes of inherited cancer susceptibility are mostly rare and characterised by variable expression and incomplete penetrance. Phenotypic variability may result from a range of causes such as allelic heterogeneity or genetic modifier effects. Another potential cause is the presence of two or more inherited cancer predisposition alleles in the same individual, an occurrence that may feasibly have a number of phenotypic consequences. Although the frequency of such occurrences might be predicted to be low, such cases have probably been under-ascertained because standard clinical practice has been to test candidate inherited cancer genes sequentially until a pathogenic mutation is detected. However, recent advances in sequencing technologies now provide the opportunity to perform simultaneous parallel testing of large numbers of inherited cancer genes.

We describe five newly identified patients who harbour pathogenic variants in multiple inherited cancer genes. Three cases involve *FLCN* mutations in combination with those in *NF1*, *TP53* and *MSH2* while combinations of *MLH1/XPA* and *BRCA2/NF1* account for the remaining cases. We also undertook a review of previously published examples to illustrate the complex genotype-phenotype relationships in these cases. We suggest that clinicians should proactively consider the likelihood of this phenomenon (referred to as multilocus inherited neoplasia alleles syndrome [MINAS]) in patients with unusual inherited cancer syndrome phenotypes. To facilitate the clinical management of novel cases of MINAS, we have established the term as a phenotypic tag in the Leiden Open Variant Database to collect information on what is likely to be an increasingly recognized cohort of such individuals.

#### P12.120

#### Novel NRAS mutation and clonal evolution detected by next-generation sequencing in a patient with juvenile myelomonocytic leukemia

*S. Lavrina<sup>1</sup>, V. Brilliantova<sup>1</sup>, I. Mersiyanova<sup>1</sup>, I. Shipitsina<sup>1</sup>, M. Kurnikova<sup>1,2</sup>, I. Kalinina<sup>1</sup>, A. Panfyorova<sup>1</sup>, E. Raykina<sup>1</sup>, A. Maschan<sup>1</sup>, M. Maschan<sup>1</sup>;*

<sup>1</sup>Federal Research Centre of Children Hematology, Oncology and Immunology named after D.Rogachev, Moscow, Russian Federation, <sup>2</sup>The Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative neoplasm of childhood with a poor prognosis. The only curative therapy is hematopoietic stem cell transplantation (HSCT), with relapse being the most common reason for treatment failure. The patient is a 5-year-old boy with a 3,5 year history of JMML. The disease had progressed to acute leukemia after low-dose chemotherapy, so the patient received allogeneic HSCT, and he relapsed after the transplantation. We performed the genetic work up at 3 points: at initial evaluation, at progression and at post-transplant relapse. DNA extracted from bone marrow was sequenced using Illumina TruSight Myeloid sequencing panel on the MiSeq platform, with following validation using Sanger sequencing. Analysis of the first point revealed a novel NRAS mutation NM\_002524: c.29\_31dupGAG (NP\_002515: p.Gly10dup) with 48% mutated allele frequencies. The point at progression revealed SETB1 c.2612T>C (p.Ile871Thr), ZRSR2 c.557+2T>G, ASXL1 c.2131insA (p.Thr711fs) and the same NRAS mutations in 24%, 96%, 45% and 49% of reads respectively. Relapse point revealed the same ZRSR2, ASXL1 and NRAS mutations in 83%, 39% and 48% of reads respectively. NRAS mutation was not found in buccal smear cells DNA samples indicating its somatic origin. NRAS is known to be one of the key genes in JMML pathogenesis, and this novel mutation is in the hot spots region for JMML, while SETBP1, ASXL1 and ZRSR2 are among targets for secondary mutations. The assessment of the mutational burden and clonal evolution in JMML can provide insight into the unfavorable outcome for the patient.

#### P12.121

#### Detection of KRAS gene mutation by liquid biopsy in patients with colorectal cancer

*P. Riera<sup>1</sup>, D. Páez<sup>2,3</sup>, M. Cornet<sup>1</sup>, A. Sebío<sup>2</sup>, A. Lasa<sup>1,3</sup>;*

<sup>1</sup>Genetics Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>2</sup>Oncology Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>U-705, CIBERER, Barcelona, Spain.

INTRODUCTION: Tumour KRAS mutational status is an important determinant of the response of metastatic colorectal cancer to targeted treatments with anti-EGFR-antibodies. Molecular analyses of somatic mutations are usually performed on tissue biopsies. However, repeat biopsies are difficult, invasive and the results may be limited by intra-tumour heterogeneity. In light of these limitations, liquid biopsy has been proved to be a noninvasive way to detect cancer mutations.

The aim of this study was to investigate the use of plasmatic cell-free tumour DNA (ctDNA) to detect and quantify KRAS mutations using a digital PCR platform in a series of clinically relevant samples.

MATERIAL AND METHODS: A total of ten colon cancer patients with a KRAS mutation in the tumour sample were included in the study. Positive and negative control samples were also analyzed.

DNA was extracted from 3ml of plasma with the QIAamp Circulating Nucleic Acid Kit (Qiagen). PCR was performed with the Droplet Digital PCR (ddPCR) from BIO-RAD.

RESULTS: The following table shows the ddPCR events obtained analyzing ctDNA of patients and the different controls. As we expected, it seems that samples at diagnosis have a higher number of KRAS mutated copies.

CONCLUSIONS: Cell-free tumour DNA (ctDNA) may be a surrogate for tumour DNA obtained from tissue biopsies. It constitutes a potential tool for identification and monitoring of KRAS somatic mutations during the course of therapy.

Patient ID	Plasma/tumour Sample	KRAS mutation	MUT events	WT events	Fractional abundance (%)
15-516	Diagnosis	G12V	119	1308	8,3
15-1252	Diagnosis	G12D	584	1572	27,1
15-2017	Diagnosis	G12V	67	430	13,5
15-2261	Diagnosis	G12D	401	2238	15,2
15-567	Follow-up	G12V	0	5036	0
15-698	Follow-up	G12V	4	3059	0,13
15-2034	Follow-up	G12D	7	2086	0,33
15-1946	Follow-up	G12V	0	642	0
15-949	Follow-up	G12D	5	1036	0,48
15-2033	Follow-up	G12D	13	818	1,6
15-1791	Plasma control	wt	0	1016	0
15-1986	Plasma control	wt	0	845	0
15-1669	Tumour control	G12V	2224	8245	21,2
15-1607	Tumour control	G12D	7870	8782	47,3

**P12.122****Genotype - Phenotype spectrum in a large Indian Li-Fraumeni Syndrome (LFS) cohort**M. Haque<sup>1</sup>, P. Kowtal<sup>1</sup>, D. Parchure<sup>2</sup>, G. Pandit<sup>1</sup>, R. Sarin<sup>1</sup>;<sup>1</sup>Advanced Centre for Treatment Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai, India, <sup>2</sup>National Institute of Immunohaematology, Mumbai, India.

**Introduction:** LFS is a rare autosomal dominant hereditary syndrome with early onset of diverse type of cancers, caused due to germline mutations in TP53 gene. Li-Fraumeni-like (LFL) syndrome has relaxed criteria for age and type of cancers. The IARC database has about 900 families with germline TP53 mutation with vast majority being from North America, Europe, Brazil, Australia and Japan. As there are no reports from South Asia, we have done comprehensive clinical & genetic characterization in a large Indian cohort.

**Materials and methods:** Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) of TP53 gene was done in 8 classical LFS, 125 LFL and 97 familial cancer probands not fulfilling LFS/LFL criteria.

**Results:** We identified 37 distinct deleterious germline TP53 mutations (2 novel) including 4 LGRs in 42/230 (18%) families. TP53 mutations were identified in 87.5% of classical LFS and 17.6% of LFL families. Surprisingly, deleterious TP53 mutations were identified in 8/97 (8%) families with familial cancers not fulfilling any LFS/LFL criteria. Comparing with the IARC database, the genotype phenotype differences in this Indian cohort include 1) Absence of hotspot codon 337 mutation; 2) high frequency of splice site, frameshift and LGRs and low frequency of missense mutations; 4) rarity of adrenocortical cancer and skin cancer.

**Conclusions:** Our study on the largest LFS/LFL Indian cohort reveals distinct clinical and genetic features and highlights the need for TP53 sequencing including MLPA for large genomic rearrangement in suspected LFS/LFL families for counseling and risk management.

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**P12.123****Colonic polyps assessment in TP53 germline mutation carriers: case series report from a tertiary cancer center in Brazil**

T. Teixeira, E. Palmero, D. Gumarães, H. Galvão;

Hospital de Câncer de Barretos, Barretos, Brazil.

**Introduction:** Li-Fraumeni syndrome (LFS), associated with germline TP53 mutation, leads to predisposition to multiple malignancies. Core tumors described in LFS are breast cancer, soft tissue and osteosarcoma, adrenocortical and central nervous system tumors. In addition, various other types of cancer are also mentioned, including colorectal cancer (CRC). Thus, annual colonoscopy is recommended for patient screening, starting from 25 years of age. However, current scientific data does not provide robust information on what type of lesions may be present at exams.

**Objective:** To describe the prevalence of polyps detected by colonoscopy in patients with LFS assessed at Hospital de Câncer de Barretos, Brazil, from November/2009 to October/2015.

**Results:** From 110 TP53 mutation carriers followed, we have assessed 58 patients (from 24 families) who underwent CRC screening (total of 95 colonoscopies). TP53 germline mutations identified in these families were: c.1010G>A (in 20/24 families), c.158G>A, c.814G>A, c.869G>A and c.997C>T (1/24 families, each). In 34 (58.6%) patients, polyps were found. Hyperplastic polyps were found in 21 (36.2%) patients, sessile serrated polyp was described in one (1.7%) patient, adenomas with low-grade dysplasia in 20 (34.5%), adenomas with high-grade dysplasia in five (8.6%), and adenocarcinoma was found in one (1.7%) patient. In 36 screened individuals, the most recent colonoscopy was performed before 50 years, and 58.3% of them already had polyps detected on exam.

**Conclusion:** Considering reports of early CRC in LFS and evidence that polyp excision reduces CRC incidence, this study corroborates the recommendation of CRC screening for LFS patients.

**P12.124****ChIP-Seq analysis of lymphocytes from Li-Fraumeni patients reveals the drastic impact on p53 DNA binding of heterozygous TP53 mutations associated with early-onset cancers**

Y. Zerdoumi, R. Lanos, I. Tournier, S. Raad, S. Baert-Desurmont, G. Bougeard, J. Flaman, T. Frebourg;

Inserm U1079, Rouen University and Department of Genetics, Rouen University Hospital, Normandy Centre for Genomics and Personalized Medicine, Rouen, France.

Li-Fraumeni Syndrome (LFS) results from heterozygous germline mutations of TP53, encoding a key transcriptional factor activated in response to DNA damage. We have recently shown, from a large LFS series including 415

TP53 mutation carriers, that the most severe form of the disease, characterized by childhood tumours, is associated with missense mutations with dominant-negative activity (Bougeard et al., J Clin Oncol 2015). Thanks to a new p53 functional assay that we developed in lymphocytes (Zerdoumi et al., Hum Mutat 2013), we found that dominant-negative missense mutations, in comparison to null mutations, drastically alter the p53 transcriptional response to DNA damage and this assay has now been used to classify 56 distinct TP53 germline alterations. To study, at the genome scale, the functional impact of heterozygous TP53 mutations on DNA binding, we performed chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-seq) analysis on lymphocytes exposed to doxorubicin, a powerful DNA damaging agent. ChIP-seq analyses of exposed control wild-type TP53 lymphocytes accurately mapped 706 p53 binding sites. New p53 binding sites were validated using a functional assay in yeast. ChIP-seq analysis of LFS lymphocytes with TP53 dominant-negative missense mutation reveals only 25 binding sites and, for these sites, the depth of the corresponding peaks was drastically reduced. Altogether, our results show that the clinical severity and predominance of TP53 dominant-negative missense mutations are explained by a global alteration of p53 binding at the genome scale and support that LFS results from a defect of the transcriptional response to DNA damage.

**P12.125****Phenotypic heterogeneity of Bannayan-Riley-Ruvalcaba syndrome - two cases from one family**A. Kutkowska-Kaźmierczak<sup>1</sup>, M. Gos<sup>1</sup>, M. Rychłowska-Pruszyńska<sup>2</sup>, E. Obersztyn<sup>1</sup>;<sup>1</sup>Department of Medical Genetic, Institute of the Mother and Child, Warsaw, Poland,<sup>2</sup>Clinic of Oncologic Surgery, Institute of the Mother and Child, Warsaw, Poland.

**Introduction:** Bannayan-Riley-Ruvalcaba syndrome (BRRS, OMIM 153480) belonging to the PTEN Hamartoma Tumor Syndrome family, is an autosomal dominant genetic disorder characterized by macrocephaly and high birth weight, benign mesodermal hamartomas (cutaneous and visceral lipomas, multiple hemangiomas and intestinal polyps), pigmented macules of the glans of penis. Intellectual delay, myopathic process and skeletal abnormalities (joint hyperextensibility, pectus excavatum and scoliosis) are observed in some patients. PTEN gene mutations located at 10q23.3 are identified in approximately 60% of BRRS patients. Individuals with BRRS and PTEN pathogenic variants have higher cancer risk and should be offered tumor surveillance protocols.

**Patients and methods:** We present phenotypic manifestation in father and daughter affected with BRRS. The child at the age of 11 with developmental delay, macrocephaly and lipomas was admitted to the genetic unit. Her father presented with high stature, macrocephaly, multinodular thyroid goiter and subcutaneous fibromas but without lipomas. Because of the suspicion of BRRS the PTEN gene was analysed using classic Sanger sequencing technique.

**Results:** The molecular analysis confirmed the diagnosis of BRRS. The mutation c.209+1G>A in intron 3 of PTEN gene was identified in both patients. We present the detailed clinical data of these two cases of BRRS in comparison with cases reported in medical literature.

**Conclusions:** The correct diagnosis of BRRS allowed for the offering of tumor surveillance protocol to both patients and for proper genetic counseling with risk assessment.

Children presenting with lipomas should be examined for other signs of BRRS and the family pedigree should always be thoroughly analysed.

**P12.126****Techniques for measuring cancer burden in liquid biopsy samples**

T. Harkins, H. Fiske, L. Kurihara, C. Schumacher, J. Laliberte, S. Sandhu, J. Irish, V. Makarov;

Swift Biosciences, Ann Arbor, MI, United States.

Liquid biopsy is a non-invasive sample source that can be utilized to assess cancer burden by measuring the tumor-derived fraction of circulating, cell-free DNA (cfDNA) from plasma. We evaluated two assays to monitor cancer burden using cfDNA: whole genome bisulfite sequencing (WGBS) and targeted amplicon sequencing for 56 oncology-related genes. We tested samples with both assays to characterize their efficacy across a broad spectrum of cancer types, stages, and treatment regimens. cfDNA was extracted from tumor-bearing patients and normal controls. To monitor methylation density, WGBS was performed using 5 ng of bisulfite-converted cfDNA with the Accel-NGS® Methyl-Seq DNA Library Kit. To detect tumor-specific mutations, 10 ng of cfDNA was used for the Accel-Amplicon™ 56G Oncology Panel. Six out of eight cancer samples demonstrated significant hypomethylation in cfDNA, ranging from 2-40% when compared to healthy controls. The 56 gene amplicon panel identified point mutations in the cfDNA of only three

samples, but which also had the highest observed hypomethylation (18-40%). For all but two cancer samples, corresponding mutations were also found in the primary tumor at allele frequencies significantly higher than in the cfDNA fraction (e.g., 22% in tumor vs. 5% in cfDNA). The three cancer samples that had primary tumor mutations that were not detected in cfDNA also had the lowest observed hypomethylation. Therefore, a correlation between hypomethylation and detection of tumor mutations in the cfDNA fraction may exist. Further studies will elucidate which assay is more sensitive at detecting tumor burden in cfDNA.

#### P12.127

#### The European Hereditary Tumour Group (formerly the 'Mallorca Group') hosts The Prospective Lynch Syndrome Database and the European Mismatch Repair cDNA Working Group and is open for all to join

P. Møller<sup>1</sup>, I. M. Frayling<sup>2,3</sup>, T. Seppälä<sup>4</sup>, I. Bernstein<sup>5,6</sup>, E. Holinski-Feder<sup>7,8</sup>, P. Sala<sup>9</sup>, D. Evans<sup>10,11</sup>, A. Lindblom<sup>12</sup>, F. Macrae<sup>13,14</sup>, I. Blanco<sup>15</sup>, R. Sijmons<sup>16</sup>, J. Jeffries<sup>2</sup>, H. Vasan<sup>17</sup>, J. Burn<sup>18</sup>, S. Nakken<sup>19,20</sup>, E. Hovig<sup>19,20,21</sup>, E. A. Rødland<sup>19</sup>, K. Thamaratnam<sup>22</sup>, W. H. de Vos tot Nederveen Cappel<sup>23</sup>, J. Hill<sup>24</sup>, J. Wijnen<sup>25</sup>, K. Green<sup>19</sup>, F. Lalloo<sup>10</sup>, L. Sunde<sup>5,26,27</sup>, M. Mints<sup>28</sup>, L. Bertario<sup>9</sup>, M. Pineda<sup>15</sup>, M. Navarro<sup>15</sup>, M. Morak<sup>7,9</sup>, L. Renkonen-Sinisalo<sup>29,30</sup>, J. Plazzer<sup>13</sup>, K. Pylyvanainen<sup>31</sup>, J. R. Sampson<sup>32</sup>, G. Capella<sup>15</sup>, J. Mecklin<sup>31,33</sup>, M. Genuardi<sup>34</sup>, G. Mösllein<sup>35</sup>, The Mallorca Group, European Hereditary Tumour Group (EHTG), International Society for Gastrointestinal Hereditary Tumours (InSiGHT), European MMR cDNA Working Group; <sup>1</sup>Research Group Inherited Cancer, Department of Medical Genetics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Institute of Medical Genetics, Cardiff University School of Medicine, Heath Park, Cardiff, United Kingdom, <sup>3</sup>Cardiff & Vale University Health Board, Cardiff, United Kingdom, <sup>4</sup>Department of Surgery, Central Finland Health Care District, Jyväskylä, Finland, <sup>5</sup>Danish HNPCC Register; Hvidovre University Hospital, Copenhagen, Denmark, <sup>6</sup>Department Surgical Gastroenterology, Aalborg University Hospital, Aalborg, Denmark, <sup>7</sup>Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Ziessmannstr. 1, Munich, Germany, <sup>8</sup>MGZ – Medizinisch Genetisches Zentrum, Munich, Germany, <sup>9</sup>Unit of Hereditary Digestive Tract Tumors IRCCS Istituto Nazionale Tumori, Milan, Italy, <sup>10</sup>Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, <sup>11</sup>Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom, <sup>12</sup>Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, <sup>13</sup>Colorectal Medicine and Genetics, The Royal Melbourne Hospital, Melbourne, Australia, <sup>14</sup>Department of Medicine, Melbourne University, Melbourne, Australia, <sup>15</sup>Hereditary Cancer Program, Institut Català d'Oncologia-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain, <sup>16</sup>Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, Nether

The Mallorca Group was formed by European members of the International Society for Gastrointestinal Hereditary Tumours (InSIGHT), to facilitate and coordinate European research on inherited colorectal cancer syndromes and issue clinical guidelines (<http://www.ncbi.nlm.nih.gov/pubmed/23408351>), and this year it has transformed into The European Hereditary Tumour Group (EHTG), open for world-wide membership (Gabriela.Moeslein@helios-kliniken.de).

The European MMR cDNA Working Group focuses on in-vitro testing for determining pathogenicity of MMR gene variants affecting splicing. (elke-holinski-feder@t-online.de). Ultimate classification of mutations ([http://chromium.lovd.nl/LOVD2/colon\\_cancer/](http://chromium.lovd.nl/LOVD2/colon_cancer/)) is made by the InSIGHT Variant Interpretation Committee. (johnpaul.plazzer@gmail.com)

The Prospective Lynch Syndrome Database (moller.pal@gmail.com), established 2013, is open for all to join. The goal is to upgrade knowledge on Lynch Syndrome (LS) from educated guesses based on retrospective series invalidated by selection biases, to prospectively obtained empirical knowledge and revise clinical guidelines accordingly. Large numbers allow reliable results: the database includes ~3,200 LS patients prospectively observed for ~25,000 years.

Results currently published (<http://www.ncbi.nlm.nih.gov/pubmed/26657901>) or in preparation include: Colonoscopy with removal of adenomas does not prevent colorectal cancer as assumed, but survival is excellent. Less than 3 years between colonoscopies was neither associated with lower incidence of colorectal cancer nor worse survival. Endometrial cancer has the highest incidence and has excellent survival. Ovarian cancer is seen early in life but most are cured. These results call for a reconsideration of clinical guidelines for identification of, and health care to, LS patients. The web-site <http://Iscarisk.org> shows the risk for cancer according to age, genotype and gender for a LS patient at any age.

#### P12.128

#### Anticipation in Lynch syndrome patients with high frequency founder mutations from R.Macedonia

M. Staninova<sup>1</sup>, N. Matevska-Geshkovska<sup>1</sup>, M. Panovski<sup>2</sup>, N. Petrushevsk<sup>2</sup>, B. Grozdanovska-Angelovska<sup>3</sup>, A. J. Dimovski<sup>1</sup>;

<sup>1</sup>Center for biomolecular pharmaceutical analyses, Faculty of Pharmacy, University St Cyril and Methodius, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>University

clinic for digestive surgery, Faculty of Medicine, University St Cyril and Methodius, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>3</sup>University clinic for radiotherapy and oncology, Skopje, Macedonia, The Former Yugoslav Republic of.

Lynch syndrome (LS) is an inherited autosomal dominant susceptibility to colorectal cancer (CRC) due to a germline mutation in mismatch repair (MMR) genes. The main aim of this study was to identify the underlying risk variants in 20 LS families from the R.Macedonia selected by the clinical Amsterdam criteria and MSI status, BRAF V600E/K mutation and MLH1 promoter methylation in tumor samples. Using targeted NGS of the coding regions of MLH1, MSH2, MSH6 and PMS2 genes we families of which 4 in MLH1 gene [p.S131X (c.392C>G), p.Thr82Ala (c.244A>G), p.Ala21Val (c.62C>T) and IVS12+8G>C (c.1409+8C>G)], 2 in MSH2 gene [MSH2 IVS14-2A>C (c.2211-2A>C) and c.68\_-72del] and one in PMS2 gene [p.Ser128Leu (c.418 G>A)]. Three new mutations [MLH1 p.S131X (c.392C>G) and p.Thr82Ala (c.244A>G and MSH2 IVS14-2A>C (c.2211-2A>C)] were detected in 5, 3 and 3 families, respectively, accounting for ~60% of the molecular defects suggesting that they are founder mutations in our population. It was noted that the disease onset was earlier for 7-10 years in each successive generation in these families, suggestive for the anticipation mechanism. Our data indicate that all new LS patients from the R. Macedonia should be initially screened for these 3 frequent variants and that CRC screening of carriers should start >10 years earlier in each successive generations.

#### P12.129

#### The LynCE study: An assesment of endometrial cancer progression markers in Lynch syndrome

M. Arias Alonso<sup>1</sup>, R. Guarch Troyas<sup>2,1</sup>, I. Chirivella González<sup>3</sup>, E. Lastra Aras<sup>4</sup>, L. Robles Díaz<sup>5</sup>, S. Moreno Laguna<sup>2,1</sup>, E. Recari Elizalde<sup>2,1</sup>, E. Urrutia Lafuente<sup>1</sup>, A. Millet Serrano<sup>3</sup>, L. Navarro Cerveró<sup>6</sup>, M. Pascual Llorente<sup>6</sup>, F. Ayuso Hernández<sup>4</sup>, D. Rueda Fernández<sup>5</sup>, P. Pérez Segura<sup>6</sup>, M. Navarro García<sup>7</sup>, M. Urioste Azcorra<sup>8</sup>, A. M. Alonso Sanchez<sup>2,1</sup>;

<sup>1</sup>Oncogenetics and Hereditary Cancer Group. IDISNA (Biomedical Research Institute of Navarre), Pamplona, Spain, <sup>2</sup>Complejo Hospitalario de Navarra, PAMPLONA, NAVARRA, Spain, <sup>3</sup>H. Clínica Universitario de Valencia, Valencia, Spain, <sup>4</sup>H. Universitario/Complejo Asistencial Burgos, Burgos, Spain, <sup>5</sup>H. Universitario 12 Octubre, Madrid, Spain, <sup>6</sup>H. Clínico San Carlos, Madrid, Spain, <sup>7</sup>IDIBELL-ICO. Insti d'Investigació Biomèdica Bellvitge-Inst Català d'Oncologia, Barcelona, Spain, <sup>8</sup>CNIO Centro Nacional Investigaciones Oncológicas, Madrid, Spain

Abnormal Immunohistochemistry (IHC) and Microsatellite Instability (MSI) have been observed both in tumoural and normal endometrial biopsies in Lynch Syndrome (LS). The potential use of these findings as markers for endometrial cancer progression was confirmed in a pilot and is being investigated in a prospective multicentre study: the LynCE.

##### Methods:

58 Lynch Syndrome (LS) carriers are now enrolled and are being followed using gynaecological/ US exam, and endometrial biopsies, accounting for a median follow-up of 10.11 months. Endometrial samples are being histologically diagnosed and evaluated for IHC, MSI, and Methylator phenotype (CIMP-MMR).

##### Results:

- All 112 gynaecological/US exams have been normal. 86/87 biopsies resulted in normal endometrium and 1/87 in endometrial cancer. All (63/63) biopsies were MSI-Stable. 32/36 showed normal while 4/36 had CIMP-MMR hypermethylation (all from a single constitutional MLH1 epimutation carrier).
- 33% (12/36) patients had abnormal IHC biopsies, 11/12 matching the underlying germline genetic defect. All abnormal IHC samples were heterogeneous, 15/16 also showing adjacent normal IHC areas.
- 3 patients progressed from normal to abnormal IHC (median=13 months) but no other clinical, ultrasound, pathological, MSI or CIMP-MMR sign accompanied this progression.

##### Conclusion:

Heterogeneous abnormal IHC is present in a subset of normal endometrium biopsies from Lynch Syndrome patients, sometimes evolving from normal IHC. Other abnormalities as MSI (in the pilot study), CIMP-MMR, and pathology changes could follow this IHC abnormality anticipating the progression to endometrial cancer.

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#### P12.130

#### Evaluation of a 25-gene panel in patients with suspected Lynch syndrome: preliminary results from the FAMOSA study

F. Balaguer<sup>1</sup>, J. Balmaña<sup>2</sup>, T. Ocaña<sup>3</sup>, I. Esteban<sup>2</sup>, A. Sanchez<sup>3</sup>, G. Llort<sup>4</sup>, R. Jover<sup>5</sup>, J. Cubilla<sup>6</sup>, M. Herranz<sup>7</sup>, L. Cid<sup>8</sup>, A. Nuñez<sup>9</sup>, M. Oruezábal Moreno<sup>10</sup>, C. Garau<sup>11</sup>, S.

Khorrami<sup>12</sup>, A. Herreros de Tejada<sup>13</sup>, R. Morales<sup>14</sup>, J. Cano<sup>15</sup>, R. Serrano<sup>16</sup>, C. Guillen<sup>17</sup>, A. Sánchez Heras<sup>18</sup>, E. Adrover<sup>19</sup>, E. Carrasco<sup>2</sup>, N. Gadea<sup>2</sup>, J. Brunet<sup>20</sup>;

<sup>1</sup>Hospital Clinic of Barcelona, CIBERehd, IDIBAPS, Barcelona, Spain, <sup>2</sup>Medical Oncology Department, Vall d'Hebron Hospital and Vall d'Hebron Institute of Oncology, Barcelona, Spain, <sup>3</sup>Hospital Clinic of Barcelona, Barcelona, Spain, <sup>4</sup>Hospital Sabadell-Parc Taulí, Sabadell, Spain, <sup>5</sup>Hospital General Universitario de Alicante, Alicante, Spain, <sup>6</sup>Complejo Hospitalario Ourense, Ourense, Spain, <sup>7</sup>Clinica Universitaria de Navarra, Pamplona, Spain, <sup>8</sup>Instituto Investigación Biomédica, Complejo Hospitalario Universitario de Vigo, Vigo, Spain, <sup>9</sup>Hospital de Mataró, Mataró, Spain, <sup>10</sup>Hospital Rey Juan Carlos, Madrid, Spain, <sup>11</sup>Hospital Son Llatzer, Palma de Mallorca, Spain, <sup>12</sup>Hospital Son Espases, Palma de Mallorca, Spain, <sup>13</sup>Hospital Universitario Puerta de Hierro, Madrid, Spain, <sup>14</sup>Hospital La Mancha Centro, Ciudad Real, Spain, <sup>15</sup>Hospital General de Ciudad Real, Ciudad Real, Spain, <sup>16</sup>Hospital Reina Sofia de Córdoba, Córdoba, Spain, <sup>17</sup>Hospital Ramón y Cajal, Madrid, Spain, <sup>18</sup>Oncología Médica, Unidad de Consejo Genético en Cáncer Hospital General Universitario de Elche, Elche, Spain, <sup>19</sup>Hospital Universitario General de Albacete, Albacete, Spain, <sup>20</sup>Heredity Cancer Program, Catalan Institute of Oncology, Girona, Spain, Barcelona, Spain.

**Introduction:** The role of multigene panels for hereditary cancer risk assessment is yet to be established. We aimed at describing the prevalence of cancer predisposition gene mutations identified by a multigene panel in individuals with suspected Lynch syndrome.

**Patients and methods:** We performed germline analysis with a next-generation sequencing 25-gene panel (Myriad myRisk™ Hereditary Cancer) using DNA from 95 patients with suspected Lynch syndrome (endometrial cancer <50 y-o and/or fulfillment of revised Bethesda criteria) from Nov-2014 through March-2015 within the FAMOSA study. We classified all identified germline variants for pathogenicity and calculated the prevalence of pathogenic mutations and variants of uncertain clinical significance (VUS). We analyzed data on patients' personal and family history of cancer.

**Results:** We included 95 patients [female: 46 (48.5%), mean age: 48.6±12]: 8 (8.5%) patients with endometrial cancer and 87 (91.5%) with colorectal cancer. Multigene panel testing identified 20 (21%) patients with Lynch syndrome mutations (8 MLH1, 7 MSH2, 4 MSH6, 1 PMS2) and 1 (1%) with a mutation in BRCA2 in a 35 y-o woman without personal/familial history of breast/ovarian cancer. In patients diagnosed with mutations in the MMR genes and prior molecular screening (n=9), two displayed MMR proficiency and five patients had a negative prior genetic result by conventional techniques.

**Conclusions:** In individuals with suspected Lynch syndrome, multigene panel testing identified unexpected high-penetrance mutations in 1% of cases. Parallel sequencing also detected a meaningful number of cases with previous false negative results.

### P12.131

#### Elucidating the molecular basis of MSH2-deficient tumors in Lynch syndrome suspected patients

G. Vargas<sup>1</sup>, E. Dámaso<sup>1</sup>, T. Pons<sup>2</sup>, J. del Valle<sup>1</sup>, S. Iglesias<sup>1</sup>, À. Velasco<sup>3</sup>, A. Solanes<sup>4</sup>, A. Valencia<sup>2</sup>, J. Brunet<sup>3</sup>, L. Feliubadaló<sup>1</sup>, C. Lázaro<sup>1</sup>, M. Navarro<sup>1</sup>, M. Pineda<sup>1</sup>, G. Capellá<sup>1</sup>; <sup>1</sup>Institut d'Investigació Biomèdica de Bellvitge-Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain, <sup>2</sup>Spanish National Cancer Research Center (CNIO), Madrid, Spain, <sup>3</sup>Institut de Investigació Biomèdica de Girona Doctor Josep Trueta-Institut Català d'Oncologia, Girona, Spain, <sup>4</sup>Institut d'Investigació Germans Trias i Pujol-Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain.

In about 55% of individuals harboring mismatch repair (MMR) deficient tumors, germline mutations or somatic methylation in MMR genes are not identified, being referred as Lynch-like syndrome (LLS). Mutations in *POLE* and *MUTYH* and double somatic events in MMR genes have been found in a proportion of these patients. The aim of this study was to elucidate the molecular basis of MSH2-deficient LLS cases by means of a comprehensive analysis of colorectal cancer (CRC) associated genes at germline and somatic level.

Eighteen LLS individuals harboring MSH2-deficient tumors were included. A customized NGS subexome panel including CRC associated genes was designed. PBL and matched FFPE DNA from available tumors (4 colorectal, 1 endometrial) were analyzed.

Predicted pathogenic germline heterozygous variants in *MSH2*, *BUB1*, *SETD2*, *FAN1* and *MUTYH* were identified in 6 of the 18 (33%) cases analyzed. The somatic analysis of tumors demonstrated the presence of: double somatic hits in *MSH2* or *MSH6* in 2 cases; and apparent loss of heterozygosity in *MSH2* locus and coexistence of double somatic mutations in other MMR and/or *POLE*/*POLD1* genes in the remaining ones. Also, somatic mutations in other cancer genes (*APC*, *AXIN2*, *BMPR1A*, *PTEN* or *BUB1B*) coexisted with the above mentioned alterations. In all, alterations putatively responsible for LLS were detected in 60% of the cases.

The evaluation of germline and somatic mutational status of CRC-associated genes by means of a subexome panel is useful for the elucidation of the molecular basis of LS-suspected cases.

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### P12.132

#### Whole gene capture analysis of 15 CRC susceptibility genes in suspected Lynch Syndrome patients

A. M. L. Jansen<sup>1,2</sup>, T. van Wezel<sup>2</sup>, S. C. Jagmohan-Changur<sup>1</sup>, D. Ruano<sup>2</sup>, H. M. van der Klijft<sup>2</sup>, B. E. W. M. van den Akker<sup>2</sup>, J. F. J. Laros<sup>1</sup>, M. van Galen<sup>1</sup>, A. Wagner<sup>4</sup>, T. G. W. Letteboer<sup>5</sup>, E. B. Gómez-García<sup>6</sup>, C. M. J. Tops<sup>3</sup>, H. F. Vasen<sup>7</sup>, P. Devilee<sup>1</sup>, F. J. Hes<sup>3</sup>, H. Morreau<sup>2</sup>, J. T. Wijnen<sup>1,2</sup>;

<sup>1</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands,

<sup>2</sup>Department of Pathology, Leiden University Medical Center, Leiden, Netherlands,

<sup>3</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands,

<sup>4</sup>Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands,

<sup>5</sup>Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands,

<sup>6</sup>Department of Clinical Genetics, University Hospital Maastricht, Maastricht, Netherlands,

<sup>7</sup>Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, Netherlands.

**Introduction:** Lynch Syndrome (LS) is caused by pathogenic germline variants in one of the mismatch repair (MMR) genes. However in up to 60% of MMR-deficient colorectal cancers no pathogenic germline MMR variant is found. Clinical management of these suspected Lynch Syndrome (sLS) cases remains difficult.

**Materials and Methods:** Using targeted next-generation sequencing, we analyzed the entire non-repetitive genomic sequence, including intronic and regulatory sequences, of 15 CRC susceptibility genes in 34 unrelated sLS patients and 11 patients with MLH1 hypermethylated tumors with a clear family history for LS. In addition, tumor DNA from 28 sLS patients was analyzed for somatic MMR variants.

**Results:** Of 1979 germline variants found in 34 sLS patients, one was a predicted pathogenic variant (MLH1 c.1667+1delG). The 11 MLH1 hypermethylated tumors were negative for germline variants in CRC susceptibility genes and for germline MLH1 hypermethylation. However, somatic DNA analysis of 28 sLS tumors identified nine (32%) cases of biallelic somatic inactivation and nine cases with (32%) one (likely) pathogenic somatic variant.

**Conclusions:** This is the first study in sLS patients to include the entire genomic sequence of CRC susceptibility genes. An underlying somatic or germline MMR gene defect was identified in ten of 34 sLS patients (29%). In the remaining sLS patients, the underlying genetic defect explaining the MMR-deficiency in tumors might be found outside the genomic regions harboring the MMR and other known CRC susceptibility genes. This work was supported by the Dutch Cancer Society under study number UL2012-5542

### P12.133

#### Classification of 8 mismatch repair variants identified in MSH2 and MSH6 genes using multifactorial likelihood analysis

M. González Acosta<sup>1</sup>, B. A. Thompson<sup>2</sup>, M. Navarro<sup>1</sup>, A. Fernández<sup>1</sup>, G. Vargas<sup>1</sup>, S. Iglesias<sup>1</sup>, A. Velasco<sup>3</sup>, A. Solanes<sup>4</sup>, J. Balmañá<sup>5</sup>, C. Lázaro<sup>1</sup>, J. Brunet<sup>6</sup>, G. Capellá<sup>1</sup>, M. Pineda<sup>1</sup>;

<sup>1</sup>Bellvitge Biomedical Research Institute - Catalan Institute of Oncology (IDIBELL-ICO), Hospitalet de Llobregat, Spain, <sup>2</sup>Huntsman Cancer Institute, University of Utah, Circle of Hope, Salt Lake City, UT, United States, <sup>3</sup>Catalan Institute of Oncology, IdIBGI, Girona, Spain, <sup>4</sup>Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Badalona, Spain,

<sup>5</sup>University Hospital Vall Hebron - Vall Hebron Institute of Oncology, Barcelona, Spain.

**Introduction:** Up to 30% of the DNA variants identified in mismatch repair (MMR) genes are variants of unknown significance (VUS). A standardized classification scheme based on quantitative and qualitative algorithms has recently been proposed for their interpretation according to the five class IARC scheme. The aim of this study was to assess the pathogenicity of MSH2 and MSH6 variants by means of multifactorial likelihood calculations.

**Patients and Methods:** Eight variants (5 missense, 2 intronic and 1 small in-frame duplication), identified in multiple families or associated to multiple tumors, were selected. Four of them had previously been classified as probably pathogenic whereas the remaining were VUS. Frequency in control population was searched in public databases. Variants were screened in DNA samples from available relatives by Sanger sequencing. Pathological information was collected. Multifactorial likelihood analysis based on estimated prior probabilities of pathogenicity, likelihood ratios for segregation and microsatellite instability in colorectal tumors was conducted as described (Thompson et al. 2013).

**Results:** None of the selected variants were reported in control population. The collected tumor data and the results of cosegregation analysis were used in likelihood ratio calculations. Posterior probability of pathogenicity resulted >0.999 for all the analyzed variants, allowing reclassification as pathogenic mutations of the 8 variants analyzed.

**Conclusions:** These results highlight the benefit of collecting segregation and tumor data for variant classification. Current multifactorial models will likely be improved when information concerning IHC patterns and MSI/IHC results of extracolonic tumors are included.

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## P12.135

### The role of 22 common susceptibility loci in phenotype variability in Lynch Syndrome patients carrying a germline PMS2 mutation

S. W. ter Broeke<sup>1</sup>, F. A. Elsayed<sup>1</sup>, L. Pagan<sup>1</sup>, M. J. W. Berends-Olderode<sup>2</sup>, E. Gomez Garcia<sup>3</sup>, H. J. P. Gille<sup>4</sup>, L. P. van Hest<sup>4</sup>, T. G. W. Letteboer<sup>5</sup>, F. H. Menko<sup>6</sup>, A. R. Mensenkamp<sup>7</sup>, T. A. van Os<sup>8</sup>, L. Spruijt<sup>8</sup>, B. J. W. Redeker<sup>9</sup>, Y. J. Vos<sup>9</sup>, A. Wagner<sup>9</sup>, C. M. Tops<sup>1</sup>, J. T. Wijnen<sup>1</sup>, T. van Wezel<sup>1</sup>, M. Nielsen<sup>1</sup>;

<sup>1</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>2</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>3</sup>Maastricht University Medical Center, Maastricht, Netherlands, <sup>4</sup>VU Amsterdam, Amsterdam, Netherlands, <sup>5</sup>University Medical Center Utrecht, Utrecht, Netherlands, <sup>6</sup>The Netherlands Cancer Institute, Amsterdam, Netherlands, <sup>7</sup>Radboud University Medical Center, Nijmegen, Netherlands, <sup>8</sup>Academic Medical Center, Amsterdam, Netherlands, <sup>9</sup>Erasmus Medical Center, Rotterdam, Netherlands.

**Background:** Lynch syndrome (LS) patients are at high risk of developing colorectal cancer (CRC). Notably, LS families show a marked intra-and inter-familial phenotype variability. This might in part be explained by common susceptibility loci, or single nucleotide polymorphisms (SNPs). Previous studies have shown conflicting results and have mainly focused on MLH1, MSH2 and MSH6 carriers. We now aim to investigate the role of these SNPs in a large cohort of PMS2 carriers.

**Methods:** Twenty-two candidate SNPs from previous Genome Wide Association studies (GWAS) were selected (rs6687758, rs6691170, rs10936599, rs1321311, rs16892766, rs6983267, rs10795668, rs3802842, rs3824999, rs4444235, rs9929218, rs4939827, rs12953717, rs10411210, rs961253, rs4925386, rs1569686, rs2736100, rs1800734, rs1799945, rs36053993, Rs1048943), which have previously been associated with (sporadic) CRC. These SNPs were genotyped in 353 PMS2 carriers ascertained from family cancer clinics (77 cases, 276 controls). Hazard ratios were corrected for familial clustering and calculated using a weighted cox regression analysis to correct for ascertainment bias. Possible effects were examined using both a dominant/recessive and an additive (per allele) model.

**Results:** We found no evidence of an association between CRC risk and cumulative number of risk alleles (HR=0.99 (95%CI: 0.90-1.08)). Moreover, none of the SNPs individually showed a risk modifying effect.

**Discussion:** There was no evidence of a risk modifying effect of twenty-two analyzed SNPs in PMS2 carriers, thus they do not appear to be of any clinical utility. Other explanations for phenotype variability that warrant further exploration include gene-environment interaction and risk modification by other genetic variants.

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## P12.136

### Lymphoid malignancies and myeloproliferative neoplasms concurrently diagnosed: the experience of a single center

M. S. Dencic Fekete, M. Perunićć-Jovanović, J. Jovanović, V. Đorđević, S. Bižić, D. Šefer, D. Antić, B. Andelić, A. Bogdanović;  
Clinic of hematology, Belgrade, Serbia.

The coexistence of lymphoproliferative and myeloproliferative neoplasms (LPN/MPN) is a rare event and has been sporadically reported in the literature. We retrospectively analyzed the clinical and biological characteristics of 7 patients with concomitant LPN and MPN - 3 chronic lymphocytic leukemia (CLL) with Polycythemia Vera (PV), 2 CLL with Essential thrombocythosis (ET), 1 diffuse large B-cell lymphoma (DLBCL) with PV and one multiple myeloma (MM) with ET - who presented to our clinic between 1995 and 2013. The average time to secondary malignancy was 62 months: LPN was diagnosed as a first disease in 1 patient (pt) and as a second in 6 patients (pts). Five patients were treated for MPN and two for LPN. Both of the patients treated for LPN achieved complete remission. After a median follow-up from MPN diagnosis of 149 months, 6 pts are still alive and 1 died. Cytogenetics was done in 6 patients: 5 pts had normal karyotype, while one pt had abnormal karyotype with derivative chromosome 18. Interphase FISH analysis was performed with CLL panel probes in three patients, showing del(13)(q14.3) in all of them. One patient with MM was analyzed with FISH probes for D13S319, TP53, IGH/FGFR3 and IGH/MAF genes and showed no rearrangements. Four patients were tested for JAK 2 mutation: 3 with PV were positive while 1 with ET was negative. This retrospective analysis showed that MPN was diagnosed before LPN in a majority of our patients. Coexistence of LPN/MPN does not appear to predict worse outcome.

## P12.137

### Mitotic kinesins are important players in the pathogenesis of malignant peripheral nerve sheath tumours

E. Terribas<sup>1</sup>, B. Gel<sup>1</sup>, J. Biayna<sup>1</sup>, J. Fernández-Rodríguez<sup>2</sup>, C. Lázaro<sup>2</sup>, E. Serra<sup>1</sup>;  
<sup>1</sup>Institut de Medicina Predictiva i Personalitzada del Càncer, Badalona, Spain, <sup>2</sup>Catalan

Institute of Oncology (ICO-IDIBELL), L'Hospitalet de Llobregat, Spain.

Around half of malignant peripheral nerve sheath tumours (MPNSTs) develop in the context of neurofibromatosis type 1 (NF1), normally progressing from pre-existing benign plexiform neurofibromas (pNFs). A genomic characterization of a set of MPNSTs confirmed the acquisition of hyperploid genomes and recurrent somatic copy number alterations for these tumours. To study the impact of these regional alterations on the transcriptome, microarray expression data was used. Mapping on the genome the differential gene expression between MPNSTs and pNFs, we identified transcriptional imbalances (TIs), genomic regions with a significant abundance of over- or underexpressed genes. Using information from TIs, some mitotic kinesin genes were identified as potential candidates for MPNST pathogenesis, which were functionally characterized in vitro using MPNST-derived cell lines.

Most of these kinesin genes were significantly overexpressed in MPNSTs in the microarray. Expression knockdown by siRNA of KIF23, involved in telophase and cytokinesis, showed that KIF23 is essential for cell survival and cell cycle progression of MPNST cell lines. Chemical inhibition of the early mitotic kinesins KIF11 and KIF10 with ispinesib and GSK923295, respectively, reduced cell viability in a set of MPNST cell lines. Moreover, according to their IC50 values, these lines were more sensitive to these compounds, especially for ispinesib, than a fibroblast cell line. Although in vivo validation is needed, our results suggest that mitotic kinesins are important players in the pathogenesis of MPNSTs, and we propose KIF11 as a new potential therapeutic target for these tumours.

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## P12.138

### Gene copy number variation analysis in male breast cancer

L. Rollán Manso<sup>1,2</sup>, I. Aurrekoetxea Rodríguez<sup>1,2,3</sup>, J. García Hernández<sup>1,3</sup>, E. Sánchez Tapia<sup>1,3</sup>, A. Pascual Rodríguez<sup>1,2,3</sup>, E. Lumbreras<sup>1</sup>, J. Hernández Rivas<sup>1,4,3</sup>, J. Cruz Hernández<sup>5,3</sup>, T. Martín Gómez<sup>6</sup>, R. Martín<sup>7</sup>, R. González Sarmiento<sup>1,2,3</sup>;

<sup>1</sup>Centro de Investigación del Cáncer, Salamanca, Spain, <sup>2</sup>Departamento de Medicina, Facultad de Medicina, Universidad de Salamanca, Salamanca, Spain, <sup>3</sup>IBSAL (Instituto de Investigación Biomédica de Salamanca), Salamanca, Spain, <sup>4</sup>Servicio de Hematología, Hospital Universitario de Salamanca, Salamanca, Salamanca, Spain, <sup>5</sup>Servicio de oncología médica, Hospital Universitario de Salamanca, Salamanca, Salamanca, Spain, <sup>6</sup>Servicio de Oncología médica, Hospital Universitario de Salamanca, Salamanca, Salamanca, Spain, <sup>7</sup>Servicio de Anatomía Patológica, Hospital Nuestra Señora de Sonsoles de Ávila, Ávila, Salamanca, Spain.

**Introduction:** Male breast cancer (MBC) is a rare disease and little is known about its etiology. So, there is limited knowledge of the disease at both genetic and molecular levels. The identification of DNA copy number variations (CNV) on MBC may results in an appropriate strategy to understand tumor pathogenesis. Therefore, the aim of this study was to analyze DNA imbalances using aCGH.

**Materials and methods:** Twelve FFPE tissue blocks of male breast cancer were used for analyze CNA utilizing Affymetrix® CytoScan 750K Array.

**Results:** All cases of male breast cancer samples displayed some chromosomal instability. An average of 179 segmented altered per cases were observed (range 16-339). Gains were most frequent than losses (1570 vs 369). The most frequent chromosome regions affected by gains were 1q21-q44, 8q11-q24, 16p13-p11 and 20q, (5/12), 17q and Xq (3/12). Deletions of 8p23-p12 and X were observed in 2 cases. Minimal common regions (MCRs) were detected on 7q11.23 (11/12), 8q21.11 (4/12), 8q24.3 (4/12), 16p11.2 (8/12), Xq21.3 (6/12) and 1q41 (5/12). MCR on 1p13.3, 6q15 21q21 and Xq13.1 (3/12) were the most frequent affected by loss.

**Conclusions:** the analysis of genomic changes by aCGH and the detection of recurrent MCRs can lead to the identification of functional important genes involved in MBC. In view of our results, male breast cancer could be considered as a different molecular entity than female breast cancer.

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## P12.139

### Mutated MCM9 is associated with predisposition to hereditary mixed polyposis and colorectal cancer in addition to primary ovarian failure

Y. Goldberg;

Hadassah Medical Center, Jerusalem, Israel.

Mutations in MCM9 encoding DNA helicase were recently shown to cause a clinical phenotype of primary ovarian failure and chromosomal instability. MCM9 plays an essential role in homologous recombination-mediated double-strand breaks repair. We describe a multiplex family with early colorectal carcinoma and mixed polyposis associated with primary hypergonadotropic hypogonadism. A combination of whole genome homozygosity

mapping as well as exome sequencing and targeted gene sequencing identified a homozygous c.672\_673delGGinsC mutation which predicts a truncated protein, p.Glu225Lysfs\*4. Our data expands the phenotypic spectrum of MCM9 mutations and suggests a link between MCM9 and inherited predisposition to mixed polyposis and early onset colorectal cancer.

#### P12.140

#### Investigation of gene expression alterations in metastatic colorectal cancer

V. P. Shubin, A. Ponomarenko, A. Tsukanov, Y. Shelygin, O. Mainovskaya, E. Rybakov, N. Pospekhova;  
Ministry of Health / State Scientific Center of Coloproctology, Moscow, Russian Federation.

Metastases of colorectal cancer (CRC) are main cause of patients' death. Data concerning gene expression alteration in primary tumors and liver metastases can serve for prognosis of disease outcome.

Methods. The materials for the study were 30 CRC tumors (T3-4N0-2cM1) and corresponding liver metastases. Expression the most important (according scientific literature data) genes CFTR, FLNA, PROM1, SFRP2, PLS3, BMI was investigated by RT- PCR. Analysis of gene expression was performed by clustering.

Results. As a result of cluster analysis all tumors and metastases were divided into 3 groups. The first cluster of tumors (6 cases) characterized by: CFTR +, FLNA-, PROM1 +, SFRP2 +, PLS3-, BMI +; the second cluster (8 cases): CFTR +, FLNA +, PROM1-, SFRP2-, PLS3 +, BMI +; the third cluster (17 cases): CFTR +, FLNA +, PROM1, SFRP2 +, PLS3 +, BMI +. The first cluster of metastases (11 cases) characterized by: CFTR +, FLNA +, PROM1 +, SFRP2-, PLS3 +, BMI +; the second cluster (8 cases) by: CFTR +, FLNA +, PROM1-, SFRP2-, PLS3 +, BMI +; the third cluster (12 cases) characterized by: CFTR +, FLNA +, PROM1-, SFRP2 +, PLS3 +, BMI +.

Conclusion. The most significant alterations of expression were detected for FLNA, PROM1, SFRP2, PLS genes in primary tumors and for PROM1, SFRP2 genes in metastases.

#### P12.142

#### Gene expression profile in melanoma cell lines carrying BRAF and NRAS mutations

P. Giménez-Xavier<sup>1,2</sup>, E. Martínez-Barrios<sup>3</sup>, J. A. Puig-Butillé<sup>2,3</sup>, G. Tell-Martí<sup>1,2</sup>, M. Potrony<sup>1</sup>, C. Badena<sup>2,3</sup>, J. Malvehy<sup>1,2</sup>, D. Benítez<sup>2</sup>, S. Puig<sup>1,2</sup>;

<sup>1</sup>Dermatology Department, Melanoma Unit, Hospital Clínic of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain, <sup>3</sup>Biochemical and Molecular Genetics Service, Hospital Clinic of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, <sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain.

Introduction: Malignant melanoma is one of the most aggressive human cancers due to its high metastatic propensity. It is well known that somatic oncogenic alterations in *BRAF* or *NRAS*, members of the MAPK (mitogen-activated protein kinase) pathway, are a common and initial event in melanocyte transformation. The oncogenic p.V600E *BRAF* mutation is found in 50% of melanomas, while mutations in *NRAS* gene are found in 20% of melanomas. Thus, identifying a gene expression profile associated with the presence of *BRAF* or *NRAS* mutations in melanoma cell-lines can provide a better understanding of their role in melanoma development and progression.

Materials and Methods: A panel of 12 melanoma cell lines derived from cutaneous melanoma or melanoma metastases was used. Cell lines were classified according to the mutational status of *BRAF* and *NRAS* genes. Analysis of whole genome expression of the cell lines was conducted by gene expression microarrays (SurePrint G3 Human Gene Expression v3 8x60K, Agilent) and differential expression was analyzed using limma. Transcripts with an adjusted p-value (FDR) <0.05 were selected.

Results: We found a genomic signature associated with p.V600E *BRAF* (36 transcripts) and another associated with *NRAS* mutations (42 transcripts). Moreover, 103 transcripts were found deregulated between p.V600E *BRAF* cell lines and *NRAS* mutated cell lines.

Conclusions: We have identified a *BRAF* and *NRAS* associated gene expression profile in a set of melanoma cell lines. These profiles can help to improve our knowledge of the role of *BRAF* and *NRAS* mutations in melanoma progression and treatment response.

#### P12.144

#### Qualitative analysis of RET protooncogene mutations responsible for Multiple endocrine neoplasia type 2 syndrome in Lithuania

L. Juodele<sup>1</sup>, D. Serapinas<sup>2</sup>, L. Kucinskas<sup>3</sup>, V. Asmoniene<sup>2</sup>, E. Juozaityte<sup>2</sup>;

<sup>1</sup>Lithuanian university of Health sciences, Kaunas, Lithuania, <sup>2</sup>Department of Genetics and molecular medicine, Lithuanian university of Health sciences, Kaunas, Lithuania, <sup>3</sup>Institute of Oncology, Lithuanian university of Health sciences, Kaunas, Lithuania.

Introduction. Multiple endocrine neoplasia type 2 (MEN2) is a rare syndrome, inherited in an autosomal dominant pattern, characterized by combination of medullary thyroid carcinoma with pheochromocytoma, tumors of the parathyroid glands and more rare tumors. The aim of the study was to determine and evaluate RET protooncogene mutations in patients with MEN2 syndrome in Lithuania.

Materials and Methods. A total of 47 unrelated patients with medullary thyroid carcinoma were enrolled into the study. Patients underwent genetic testing by DNA sequencing, detecting germline mutations causing MEN2 syndrome.

Results. RET protooncogene mutations causing MEN2 syndrome were detected in 8 patients of the 47 medullary thyroid carcinoma cases - 17.02% (CI 95% 6.4 - 27.7%). The most frequent RET protooncogene mutations (in 611 (n=2), 618(n=1), 634 (n=2) codons) were detected to cause MEN2A syndrome (62.5%), less frequent mutation in 918 codone (n=2) that caused MEN2B syndrome, most rare - familial medullary thyroid carcinoma syndrome causative mutation in 791 codone (n=1). In patients with detected MEN2 syndrome causative mutations in RET protooncogene, medullary thyroid carcinoma manifested in younger age - 29.8 ±16.1 years, comparing with sporadic cases - 49.5±10.7 years (p<0.05).

Conclusions. In unrelated patients with medullary thyroid carcinoma detected frequency of probands and germline RET protooncogene mutations causing MEN2 syndrome was 17.02 (CI 95% 6.4 - 27.7%).

#### P12.145

#### Epigenetic inactivation of tumor suppressor genes correlates with tumor recurrence in histologically benign meningiomas

T. San-Miguel<sup>1</sup>, L. Navarro<sup>1</sup>, J. Megías<sup>1</sup>, L. Muñoz-Hidalgo<sup>2</sup>, R. Gil-Benso<sup>1</sup>, M. Cerdá-Nicolás<sup>1</sup>, C. López-Ginés<sup>1</sup>;

<sup>1</sup>Department of Pathology, University of Valencia, Valencia, Spain, <sup>2</sup>INCLIVA, Valencia, Spain.

Meningiomas are the most frequent tumors of the central nervous system. Histologically, they are divided in three grades according to their aggressiveness. Grade I tumors are the most common, and they are usually slow-growing tumors, curables by surgery. Nevertheless, in absolute numbers, they cause the majority of recurrences. Despite cytogenetic and genetic approaches for meningioma progression, prediction of recurrence in histologically benign meningioma remains a challenge.

The aim of this work was to characterize epigenetic changes in recurrent versus non-recurrent grade I meningiomas. We completed the study comparing primary meningiomas with tumor recurrences, in order to determine characteristic changes in progressed tumors. Formalin-fixed paraffin-embedded specimens were used for DNA extraction and studied by Methylatin-Specific Multiplex Ligation-dependent Probe Amplification with ME001-C2 tumor suppressor genes (TSG) kit (MRC-Holland). This technique provides promoter hypermethylation information of several genes in just one reaction.

The study revealed a statistically significant increase in the global number of hypermethylated TSG in grade I recurrent tumors in a similar way that happens in the recurrences analyzed versus primary tumors. Specifically, epigenetic changes of MLH1, RASSF1A and CDKN2B correlated to the tendency to recur. In addition, the epigenetic landscape of grade I-recurrent tumors resembled to the one found on recurrences.

Our results suggest that TSG hypermethylation is an early event in the acquisition of aggressiveness in benign meningioma and the role of these genes that are implicated in DNA-reparation, neoangiogenesis and cell-cycle control should be further studied.

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## P12.146

### Evaluation of Microsatellite Instability in Tumor and Tumor Marginal Samples of Sporadic Colorectal Cancer Using Mononucleotide Markers

J. Nouri Nojadeh, E. Sakhinia, S. Hashemzadeh, S. Behrouz Sharif;  
Tabriz University of Medical Science, Tabriz, Iran, Islamic Republic of.

**Background:** Microsatellite instability in tumor DNA is defined as the presence of alternate sized repetitive DNA sequences that are not seen in the corresponding germline DNA. Microsatellite instability (MSI) is a molecular phenotype due to a defective DNA mismatch repair system. The presence of MSI is found in sporadic colon, gastric, sporadic endometrial and the majority of other cancers. Determination of MSI status in CRC has a clinical use in the form of identifying patients with germline defects predisposing to MMR-deficiency. Additionally, MSI status has prognostic and therapeutic implications, due to MSI CRCs typically exhibiting a better prognosis. For these reasons microsatellite instability analysis is becoming more important in detecting sporadic primary tumors.

**Material and methods:** In this study we investigated tumoral DNA and tumor marginal DNA of 50 sporadic CRC patients who have not received chemotherapy. Five mononucleotide markers, BAT-25, BAT-26, NR-21, NR-22 and NR-27 were used as a pentaplex PCR panel to evaluate for microsatellite instability status.

**Results:** Our primary finding showed that MSI was detected in about 40% of specimens. Instability was observed in the tumoral DNA compared to the DNA from the normal DNA sample. Our primary results showed that the frequency of instability NR-21, BAT-25 and NR-27 markers more than other markers and its frequency almost were similar.

**Conclusion:** Our study showed NR-21, BAT-25 and NR-27 were the most useful markers for diagnosing sporadic CRC. Therefore these markers have shown promise in their potential use for determining MSI status in patients with sporadic colorectal cancer.

## P12.147

### The phenotype of the POLE germline mutation p.(Val411Leu) overlaps with constitutional mismatch repair deficiency syndrome

K. Wimmer<sup>1</sup>, A. Beilken<sup>2</sup>, R. Nustede<sup>3</sup>, T. Ripperger<sup>4</sup>, B. Ure<sup>5</sup>, D. Steinmann<sup>5</sup>, J. Zschocke<sup>1</sup>, C. Fauth<sup>1</sup>, C. Kratz<sup>2</sup>;

<sup>1</sup>Division Human Genetics, Medical University Innsbruck, Innsbruck, Austria, <sup>2</sup>Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany, <sup>3</sup>Department of Surgery, Childrens Hospital, Hannover Medical School, Hannover, Germany, <sup>4</sup>Institute of Human Genetics, Hannover Medical School, Hannover, Germany, <sup>5</sup>Department of Radiotherapy and Special Oncology, Hannover Medical School, Hannover, Germany.

There are three adenomatous polyposis and colorectal cancer (CRC) susceptibility syndromes that result from constitutional DNA repair defects. The phenotypes of the constitutional base excision repair defect, MUTYH-associated polyposis (MAP), and of impaired exonuclease function of replicative polymerases, polymerase proofreading-associated polyposis (PPAP), are characterized by multiple adenomas and/or carcinoma usually diagnosed in adulthood. Constitutional mismatch repair deficiency (CMMRD) confers a more penetrant phenotype with a high risk for childhood or adolescence onset of polyposis and CRC as well as for other malignancies. Despite the presence of multiple café-au-lait macules (CALM) and one pilomatricoma, which serve as diagnostic signposts for CMMRD in young cancer patients, this diagnosis was ruled-out by gMSI and mutation analysis of the four MMR genes in a 14-year-old boy with polyposis and a rectosigmoid cancer expressing all four MMR genes. The only functionally-relevant variation found by analysis of eight polyposis genes is p.(Val411Leu) in POLE (NM\_006231.3). This somatic hot-spot mutation recurrently found in 'ultramutated' sporadic CRC was never before observed as germline alteration. It impairs Pole's exonuclease activity and, therefore, is considered responsible for the phenotype of this youngest reported CRC patient with PPAP. This PPAP case reminiscent of CMMRD raises the question of a skin phenotype that is common to different types of constitutional DNA repair defects and results from postzygotic mutations secondary to the repair defect. Together with anecdotic reports of multiple CALM and pilomatricomas in MAP patients, this case supports this notion and should prompt investigations for skin features in MAP and PPAP patients.

## P12.148

### Mitochondrial DNA mutational changes in glioma

M. M. González<sup>1</sup>, C. Santos<sup>1</sup>, A. Ramos<sup>1</sup>, M. Cos<sup>2</sup>, C. F. Alarcón<sup>3</sup>, J. J. Acebes<sup>3</sup>, M. P. Aluja<sup>1</sup>;

<sup>1</sup>Unitat d'Antropologia Biològica, Departament BABVE, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain, <sup>2</sup>Servei de Neuro-radiologia, Institut de Diagnòstic per la Imatge, Centre Bellvitge, Hospital de Bellvitge, Barcelona, Spain, <sup>3</sup>Servei de Neurocirurgia, Ciutat Sanitària i Universitària de Bellvitge, Hospital de Bellvitge, Barcelona, Spain.

Mitochondrial anomalies have been linked to carcinogenic processes. The production of reactive oxygen species (ROS) increases the probability of suffering oxidative damage and, consequently, the accumulation of mitochondrial DNA (mtDNA) mutations, affecting conserved positions probably contributing to the development of disease. This study aims to characterize the whole mitochondrial genome in patients affected by glioma. A total of 27 glioma cancer patients were studied. Brain tumour and blood samples were collected for each patient and 10 brain samples from healthy individuals were added to the study. mtDNA was sequenced using Nextera XT kit with MiSeq sequencer from Illumina. Comparing tumour and blood samples from the same patient, results show that the vast majority of the mutations were present in both tissues (87.02%), assuming a high representation of germinal mutations. Somatic mutations in brain tumours (12.98%) are mainly in heteroplasmy (70.59%). The comparison between brain tumour samples and brain samples from healthy individuals shows that both present a low percentage of mutations in heteroplasmy and their frequencies are very similar (18.32% in brain tumour samples; 18.18% in brain samples from healthy individuals). The study could contribute to understanding the implication of mtDNA in cancer development, specifically its role in glioma tumorigenesis.

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## P12.149

### The genomic landscape of mitochondrial DNA mutations in chronic myeloid leukaemia

I. S. Pagani<sup>1,2</sup>, C. H. Kok<sup>1,3</sup>, V. Saunders<sup>1</sup>, J. Goyne<sup>1</sup>, J. McLean<sup>1</sup>, M. VanderHoek<sup>1</sup>, S. Heatley<sup>1</sup>, A. P. Schwarer<sup>4</sup>, T. P. Hughes<sup>1,3,5</sup>, D. L. White<sup>1,3</sup>, D. M. Ross<sup>1,5,6</sup>;

<sup>1</sup>South Australian Health and Medical Research Institute, Adelaide, Australia, <sup>2</sup>The University of Milan, Milano, Italy, <sup>3</sup>School of Medicine, University of Adelaide, Adelaide, Australia, <sup>4</sup>Box Hill Hospital, Melbourne, Australia, <sup>5</sup>Department of Haematology, SA Pathology, Adelaide, Australia, <sup>6</sup>Flinders University and Medical Centre, Adelaide, Australia.

**Introduction:** Genomic instability in BCR-ABL1-positive chronic myeloid leukaemia (CML) is reported to be associated with increased reactive oxygen species (ROS). The mitochondrial (mt) genome is susceptible to ROS-induced mutations due to oxidative stress in the mitochondrion and limited DNA-repair mechanisms.

**Rationale:** To describe the mt-genomic landscape in CML, and to identify diversity in the apparently uniform leukemic clone.

**Methods:** We performed targeted next-generation sequencing on mt-genome in 27 CML patients at diagnosis and after 12 months of therapy. Patients were selected as good (n=15) or poor (n=12) responders based on achievement of major molecular response (BCR-ABL1<sup>IS</sup> ≤0.1%) at 12 months. Mesenchymal stem cells or hair follicles were used to exclude germline polymorphisms.

**Results:** We identified 87 somatic mutations at diagnosis, in 14/15 good responders and in 9/12 poor responders. The number of somatic mutations per patient tended to be higher in good responders than in poor responders. All somatic mutations identified at diagnosis were heteroplasmic, reverting to wild type at follow up. Seven good responders and 5 poor responders had new heteroplasmic mutations detected in follow-up samples. The distribution of mutations across the mt-genome did not differ between good and poor responders, and did not show an excess of G>T transversions that are considered the hallmark of ROS-induced damage.

**Conclusions:** MtDNA mutations are common in CML, and identify clonal diversity both at diagnosis and follow up. Mitochondrial mutations are not directly related to ROS damage or clinical outcome. The significance of mutations in remission requires further study.

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## P12.150

### Molecular characterization of constitutional MLH1 epimutations

E. Dámaso<sup>1</sup>, A. Castillejo<sup>2</sup>, M. Arias<sup>3</sup>, G. Vargas<sup>1</sup>, P. Mur<sup>1</sup>, M. Genuardi<sup>4</sup>, D. Rueda<sup>5</sup>, Á. Alonso<sup>3</sup>, J. Soto<sup>2</sup>, M. Pineda<sup>1</sup>, G. Capellá<sup>1</sup>;

<sup>1</sup>Bellvitge Biomedical Research Institute - Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain, <sup>2</sup>Elche University Hospital, Elche, Spain, <sup>3</sup>Complejo Hospitalario de Navarra, Pamplona, Spain, <sup>4</sup>Catholic University of the Sacred Heart, Rome, Italy, <sup>5</sup>Doce de Octubre University Hospital, Madrid, Spain.

**Introduction:** Constitutional epimutations in MLH1 have been identified in a subset of Lynch syndrome patients (0-2%). Little is known about their mechanistic basis and consequently its inheritance pattern. The aim of this study was to perform a genetic and epigenetic characterization of constitutional MLH1 epimutations.

**Methods:** Twelve patients carriers of constitutional MLH1 epimutations were recruited (8 previously reported). Mutational analysis of MLH1 promoter and intron 1 was performed by Sanger sequencing. The presence of structural alterations was evaluated using a customized array with 15K probes (Agilent Technologies), surrounding MLH1 gene. Clonal bisulfite sequencing of the promoter was used to determine the methylated allele. Inheritance pattern was determined by haplotype and MS-MLPA analyses in probands' first-degree relatives. Global methylome analysis was performed using Infinium 450K array (Illumina).

**Results:** The variant c.-234.-236del was identified in one of 12 cases analyzed and rearrangements in the locus surrounding MLH1 were found in 4 of the 11 analyzed patients. Methylation was confined to a single allele in two analyzed cases, associated to MLH1 transcriptional inactivation. Subsequent methylation and haplotype analyses in relatives revealed intergenerational erasure. Global methylome analysis in the 12 cases revealed that MLH1-EP-M2AIP1 promoter CpG island is the most differentially methylated region of MLH1 epimutants when compared with 21 MLH1 mutation carriers.

**Conclusions:** Structural alterations are found in a significant proportion of MLH1 constitutional epimutations. Further studies are needed to assess its putative causal relationship. Refined molecular characterization is needed to elucidate their mechanistic basis and heritability.

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#### P12.151

#### A novel mutation in hMLH1 causing positive hMLH1 immunohistochemical staining, but defective binding to and loss of hPMS2

A. Stella, M. Patruno, D. C. Loconte, S. Di Tommaso, C. Ranieri, C. Simone, D. Varvara, R. Bagnulo, P. Lastella, N. Resta;  
Division of Medical Genetics- Department of Biomedical Sciences and Human Oncology, University Of Bari, Bari, Italy.

**Introduction:** The advent of NGS has caused the identification of novel variants in Lynch syndrome patients, whose clinical consequences are difficult to predict especially when associated with conflicting results of immunohistochemistry analyses. We here report a novel mutation in hMLH1 which, albeit leading to normal hMLH1 expression impairs its ability to bind to hPMS2 causing PMS2 lack of expression

**Materials and Methods:** The index case was represented by a 23 years old male diagnosed with a pT3N1M1 colon adenocarcinoma. DNA isolated from PBL was deep sequenced using an Ion AmpliSeq custom panel on an Ion Torrent PGM. IHC for MLH1, MSH2, PMS2 and MSH6 was done according standard methods. In silico analysis was performed using the following software prediction tools: Variant Effect Predictor (VEP), PredictProtein, and i-Mutant.

**Results:** NGS analysis revealed the presence of a c.122C>T, p. D41V in the hMLH1 coding sequence as the most likely pathogenic variant. IHC demonstrated positive staining for MLH1, MSH2, MSH6 but not for PMS2. In silico analysis showed the following results: VEP returned a moderate score for the pathogenicity of this variant with all predictions from SIFT, Polyphen, and MutationTaster either deleterious or probably damaging. PredictProtein computed for the mutant protein the loss of five binding sites, the reduction in length of additional two, while three novel binding sites were gained. Finally, i-mutant predicted a stability decrease for the mutant protein

**Conclusions:** The identified variant falls in the ATPase domain where 50% of missense variants cluster despite accounting for less than 25% of aminoacid sequence.

#### P12.152

#### Unclassified sequence variants: a challenge for molecular oncogenetic diagnostics

L. Negura<sup>1</sup>, A. Chicos<sup>1</sup>, D. Azoicai<sup>1</sup>, A. Negura<sup>2</sup>;  
<sup>1</sup>Gr. T. Popa Medicine and Pharmacy University, Iasi, Romania, <sup>2</sup>Alexandru Ioan Cuza University, Iasi, Romania.

Oncogenetics was developed 20 years ago as a clinical and psychological follow-up addressing to patients and families with hereditary risk of cancer. Oncogenetic approach developed together with the identification of predisposition genes to breast, ovarian or colorectal cancer, and with the progress in molecular diagnosis abilities. Today, the only molecular technique accepted and validated for oncogenetic diagnosis is Sanger sequencing of all exons and exon-intron boundaries for the known predisposition genes. When completely sequencing and decrypting thousands of DNA nucleotides, many sequence variants are identified, most of them being single nucleotide polymorphisms. Current practice classifies sequence variants in a 5-levels frame, from deleterious pathogenic to polymorphic benign. Unfor-

tunately, more than 40% of the sequence variants found in routine analysis are of unclear pathogenicity. While several biochemical, molecular, genetics and *in-silico* analysis may help in interpreting such variants, there still rest an important percentage with unclear clinical value.

Here we present several distinct unclassified variants (UV) and how they could be interpreted for a clinical purpose. Case 1: Validating a UV to benign by *in-silico* or *in-trans* analysis; Case 2: Validating a UV to benign by functional tests; Case 3: doubts persisting when the result is level 2 "probably benign"; Case 4: Segregation and linkage analysis for results level 4 "probably benign"; Case 5: The ultimate challenge - the level 3 absolute unclassified variant.

Interpreting such variants, as well as communicating the diagnosis results both to patients, families or clinicians, are the biggest challenges for present molecular diagnosis.

#### P12.153

#### Investigation of gene expression of myeloma cells in bone marrow of multiple myeloma patients by transcriptome analysis

M. Sariman<sup>1</sup>, S. Sirma Ekmekci<sup>1</sup>, N. Abaci<sup>1</sup>, A. Cakiris<sup>1</sup>, F. Pacal Percin<sup>1</sup>, D. Ustek<sup>1</sup>, M. Ayer<sup>2</sup>, M. Yenerel<sup>3</sup>, S. Besisik Kalayoglu<sup>3</sup>, K. Cefle<sup>4</sup>, S. Palanduz<sup>2</sup>, S. Ozturk<sup>2</sup>;

<sup>1</sup>Istanbul University, Research Institute of Experimental Medicine Department of Genetics, Istanbul, Turkey, <sup>2</sup>Haseki Education and Research Hospital, Department of Hematology, Istanbul, Turkey, <sup>3</sup>Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine Department of Hematology, Istanbul, Turkey, <sup>4</sup>Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Turkey.

**Introduction:** Multiple Myeloma (MM) is the hematological disease characterized by transformation of B cells into malignant cells. Our aim of the study was to investigate transcriptome profiles in bone marrow myeloma cells by next generation sequencing technology.

**Method:** In this study we performed RNA-Seq using the Ion Torrent PGM platform to compare the transcriptome profiles of untreated four MM patients and four healthy donors. Myeloma cells (CD38+, CD138+, CD19+, CD56+) and healthy B cells (CD38+, CD138+, CD19+, CD56-) from bone marrow were selected using cell surface marker in FACSaria II Cell Sorter. Transcriptome libraries were prepared from rRNA depleted RNA extracted from pool of myeloma cells and healthy donors B cells. These libraries were sequenced and aligned the reads to the human genome (GRCh37/hg19) and mapped sequences to the RefSeq database.

**Result:** Here, we characterize transcriptional profiles in MM patients. In addition, 18806 transcript (17760 of transcripts were reported), 490 deletion, 1397 insertion, 8402 single nucleotide variants 415 reported fusion gene, 983 novel fusion gene were detected. The genes found differentially expressed underwent Gene Set Enrichment Analysis by MSigDB. We identified significant overlap between EEF1A1, UBC, UBB, CALR, CXCR4, JUND, FOS, PIM2, JUN, GAPDH, HSP90B1 that was previously reported upregulation of MM.

**Conclusion:** We were determined some variations and different mRNA expression pattern in some previous reported genes that especially in ubiquitin-proteosomal pathway. Possible candidate genes detected in this study should be confirmed with real time PCR and sanger sequencing in a large groups of patients.

#### P12.154

#### Clonal evolution in bone-marrow cells of patients with myelodysplastic syndromes (MDS) and deletion 5q

K. Slobodanova<sup>1</sup>, Z. Zemanova<sup>1</sup>, H. Lhotska<sup>1</sup>, M. Novakova<sup>1</sup>, M. Belickova<sup>2</sup>, J. Brezinova<sup>2</sup>, I. Sarova<sup>1,2</sup>, S. Izakova<sup>1</sup>, L. Litzcova<sup>1</sup>, A. Berkova<sup>1</sup>, A. Jonasova<sup>3</sup>, J. Cermak<sup>2</sup>, K. Michalova<sup>1,2</sup>;

<sup>1</sup>Center of Oncocytogenetics, IMBLD, General University Hospital and 1st Faculty of Medicine, Charles University in Prague, Prague 2, Czech Republic, <sup>2</sup>Institute of Hematology and Blood Transfusion, Prague 2, Czech Republic, <sup>3</sup>First Medical Department, General University Hospital and First Faculty of Medicine, Charles University in Prague, Prague 2, Czech Republic.

The most common recurrent chromosomal change in bone-marrow cells of patients with myelodysplastic syndromes (MDS) is interstitial deletion of the long arm of chromosome 5. Finding of the sole del(5q) is associated with good prognosis. Acquisition of additional aberrations during clonal evolution usually leads to rapid disease progression. The aim of our study was to thoroughly analyze bone-marrow cells of five MDS patients with sole del(5q) in the original clone and with successive linear or divergent clonal evolution proved in ancestral cell clones.

Bone-marrow samples were analyzed by conventional G-banding, I-FISH/mFISH/mBAND (MetaSystems) and aCGH/ SNP microarray (Illumina). To detect TP53 mutations, NGS (GS Junior system, Roche) was used.

In all five patients, interstitial del(5q), extending between 5q13.4 and

5q35.2, encompassed commonly deleted regions 5q31 and 5q33. Clonal evolution concerning 5q included the duplication of deleted chromosome 5 in one sample and new translocation t(X;5)(q13;q12) in the other one. However, additional chromosomal changes acquired during clonal evolution were identified across the whole genome. Most frequently affected chromosome arms were 11q (9x), 11p (6x) and 17p (6x). In four of five patients copy number neutral loss of heterozygosity (CN-LOH) of 17p and corresponding missense mutations of TP53 gene (17p13.1) were found. The evaluation of primary and secondary aberrations is important for identification of mechanisms involved in clonal evolution of pathological cells, which can contribute to better understanding of the disease progression and is indeed very important for prognosis of the patients.

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#### P12.155

#### The expression analysis of F2R gene in JAK2V617F mutation positive Polycythemia Vera (PV)

I. Uslu<sup>1</sup>, H. Hekimoglu<sup>1</sup>, A. S. Yavuz<sup>2</sup>, S. Sözer Tokdemir<sup>1</sup>;

<sup>1</sup>Department of Genetics, Institute of Experimental Medicine, İstanbul University, İstanbul, Turkey, <sup>2</sup>Istanbul Faculty of Medicine, Internal Medicine Division, Department of Hematology, İstanbul University, İstanbul, Turkey.

Introduction: Myeloproliferative neoplasms (MPN) are stem cell originated diseases associated with high risk of thrombosis. 50- 98% of patients carry JAK2V617F. F2R gene, has a role as a receptor regulates thrombotic response. The aim of this study is to analyze the F2R gene expression levels both in CD34+ and mononuclear cells (MNC) of patients related with the JAK2V617F.

#### Materials-Methods;

MNCs were isolated from peripheral blood of 5 patients who were diagnosed with PV and one cord blood (CB). The cells were stained and sorted against CD34. JAK2V617F were investigated in CD34+ and MNCs using allele-specific nested PCR. F2R gene expression evaluation were performed by real-time RT-PCR in MNCs, CD34+ and HEL. The analysis was performed by using 2-ΔCT.

Results: The analysis for JAK2V617F in patients revealed that four were heterozygous JAK2V617F and one was wild type(wt) JAK2 allele. F2R gene expression analysis of CD34+ between patients and CB revealed the 60.1 ± 49.1 fold increase whereas the MNCs of the same patients has 4.5± 5.7 fold increase compared to CB. There was no difference between JAK2V617F/+ patients regarding to F2R. HEL, homozygous for JAK2V617F, has 4.1 fold increase.

Conclusion: The analysis of the F2R gene expression has revealed that the CD34+ of patients has statistically significant increase compared to controls. This is the first investigation in literature demonstrating the role of F2R in MPN related with the JAK2V617F. The further studies are needed to address the exact role of F2R and its relationship between disease and pathophysiology.

#### P12.156

#### Association between NBN mutation and lung cancer - a new risk factor

J. S. Nowak<sup>1</sup>, E. Kałużna<sup>1</sup>, J. Rembowska<sup>1</sup>, I. Ziółkowska-Suchanek<sup>1</sup>, B. Świątek-Kościelna<sup>1</sup>, P. Gabryel<sup>2</sup>, W. Dyszkiewicz<sup>3</sup>;

<sup>1</sup>Institute of Human Genetics, Poznan, Poland, <sup>2</sup>Department of Thoracic Surgery University of Medical Sciences, Poznan, Poland, <sup>3</sup>Department of Thoracic Surgery University of Medical Sciences, Poznan, Poland.

The NBN gene, also known as NBS1, is located on chromosome band 8q21.3 and encodes 754-amino acids protein called nibrin. This protein is a member of the MRN nuclear complex, and is involved in numerous cell processes essential for maintaining genomic stability. Heterozygous variants in the NBN gene, including p.I171V, c.657del5 and p.R215W, have been described as a risk factors for the development of several malignancies. However, there is no report regarding the association of these mutations with lung cancer thus far. Therefore, the present study aimed to evaluate whether there is an association between heterozygous p.I171V, c.657del5 and p.R215W variants of the NBN gene and the risk of developing lung cancer.

The frequency of these variants was estimated in a group of 453 adults diagnosed with non-small cell lung cancer and in healthy controls (2,400 for p.I171V, 2,090 for c.657del5 and 498 for p.R215W). The p.I171V was assessed by restriction fragments length polymorphism analysis of polymerase chain reaction (PCR) products, using MspI (MfeI) restriction enzyme, whereas the c.657del5 and p.R215W were assessed by PCR-single-strand conformation polymorphism method.

A significantly increased risk of developing lung cancer was observed for the p.I171V variant, which was present in 17 (3.75%) of the 453 cases of lung cancer and in 12 (0.5%) of the 2,400 healthy individuals (odds ratio, 7.759; P<0.0001).

The results obtained indicated an association between the p.I171V mutation and the development of lung cancer. Therefore, this variant may be considered as a risk factor for non-small cell lung cancer.

#### P12.158

#### Neuroendocrine tumors in 483 children in the Netherlands: a descriptive study of tumor characteristics and genetic predisposition

I. J. Diets<sup>1</sup>, I. Nagtegaal<sup>2</sup>, J. Loeffen<sup>3</sup>, E. Waanders<sup>1</sup>, I. de Blaauw<sup>4</sup>, N. Hoogerbrugge<sup>1</sup>, M. Jongmans<sup>5</sup>;

<sup>1</sup>Department of Human Genetics, Radboud University Medical Centre and Radboud Institute for Molecular Life Science, Nijmegen, Netherlands, <sup>2</sup>Department of Pathology, Radboud University Medical Centre, Nijmegen, Netherlands, <sup>3</sup>Department of Pediatric Oncology and Hematology, Sophia Children's Hospital, Erasmus Medical Centre, Rotterdam, Netherlands, <sup>4</sup>Department of Surgery - Division of Pediatric Surgery, Amalia Children's Hospital, Radboud University Medical Centre, Nijmegen, Netherlands.

Introduction: Neuroendocrine tumors (NETs) are rare in children. Genetic predisposition for these tumors has been described, but only limited data are available. We conducted a large cohort study to specify tumor and pa-

Table1. Mutations determined by resequencing and patient characteristics							
Patient No	Age/Gender	Mutation	Predicted Change	ClinVar	HGMD	Leiden Open Variation Database	Clinical findings additional to café au lait spots
1	7/Male	c.1541_1542delAG	p.Gln514Argfs*43	rs267606600, pathogenic	CM1110944	Pathogenic	Unknown
2	4/Male	c.6709C>T	p.Arg2237Ter	Not Reported	CM000815	Pathogenic	Unknown
3	8/Male	c.5389C>T	p.Arg1947Ter	rs137854552, pathogenic	CM900173	Pathogenic	surgery due to intestinal obstruction at age 3 neurofibroma in the left eye T2 flair hyperintense signals in basal ganglia in cranial MRI
4	2/Male	c.6756+1G>T	splice donor site changes	Not Reported	CS982281	Pathogenic	facial asymmetry micrognathia
5	13/Female	c.7395-2A>G	splice acceptor site changes	Not Reported	CS098053	Pathogenic	multiple hamartomas in the brain
6	7/Male	c.3113+1G>C	splice donor site changes	Not Reported	Not Reported	Pathogenic	nodular lesions with high signal intensities on both corpus callosum
7	41/Male	c.4867G>C	p.Asp1632His	Not Reported	Not Reported	Pathogenic	neurofibromas Strong contrast regions in femur MRI peripheral nerve sheath tumor signs
8	1/Female	c.4802delT	p.Leu1601Cysfs*2	Not Reported	Not Reported	Not Reported	lower right tibia break
9	3/Female	c.5630T>A	p.Leu1877Ter	Not Reported	Not Reported	Not Reported	unknown
10	6/Male	c.7907+1_7907+4del	splice donor site changes	Not Reported	Not Reported	Not Reported	lipoma in the waist neurofibroma on the neck and on the right arm

tient characteristics of children with a NET. We aimed to investigate the role of genetic predisposition in the etiology of pediatric NETs by collecting data of second primary malignancies and comorbidities suggestive of genetic predisposition.

**Patients and methods:** Using the nationwide pathology database PALGA, we collected patient- and tumor data of all children diagnosed with a NET in the Netherlands between 1991 and 2013 (N=483).

**Results:** The incidence of NETs in children in the Netherlands is 5.40 per one million per year. The majority of NETs were appendiceal tumors (N=441; 91.3%). In only 16 patients (3.3%) either locoregional lymph node or distant metastases were present. Three patients with pancreatic NETs were diagnosed with the known tumor predisposition syndromes Von Hippel Lindau disease (N=2) and Multiple Endocrine Neoplasia syndrome type 1 (N=1). One additional patient with NETs in the stomach and pancreas had MEN-1 syndrome. In one patient with an appendiceal NET Familial Adenomatous Polyposis was diagnosed. A role of genetic predisposition was suggestive in several others, e.g. in patients with second primary malignancies (N=6, 1.2%) or multiple NETs (N=5, 1.0%).

**Conclusion:** We identified a significant number of patients with a confirmed or suspected tumor predisposition syndrome and show that pediatric pancreatic NETs in particular are associated with cancer prone syndromes.

#### P12.160

#### NF1 and SPRED1 mutational spectrum of 1.100 unselected patients suspected of having Neurofibromatosis Type 1

**B. Campos<sup>1</sup>, O. Abad<sup>2</sup>, J. Gardenyes<sup>2</sup>, P. Fabregas<sup>2</sup>, E. López-Toledano<sup>2</sup>, V. Volpint<sup>2</sup>,**  
<sup>1</sup>Reference Laboratory Genetics, L'Hospitalet de Llobregat, Spain, <sup>2</sup>IDIBELL, L'Hospitalet de Llobregat, Spain.

**Introduction:** Neurofibromatosis type 1 (NF1) [MIM 162200] is a common dominant autosomal disorder, affecting 1 in 3500 individuals and caused by mutations in the *NF1* gene at 17q11.2. Legius syndrome [MIM 611431], caused by mutations in *SPRED1* gene (15q13.2), is characterized by clinical manifestations that overlap NF1, as café-au-lait spots and freckling. **Materials and Methods:** We screened 1.100 unrelated NF1 patients for mutations in *NF1* and *SPRED1* genes, using direct cDNA sequencing complemented with MLPA analysis. **Results:** We identified 710 mutations in *NF1* and 19 in *SPRED1*. Among the 710 changes in *NF1*, 184 (26%) nonsense, 180 (25%) were frameshift mutations, 173 (24%) splice errors, 105 (15%) were missense and 12 (2%) were in frame amino acid deletions/insertions. Furthermore, using MLPA approach we found 55 mutations (8%): 29 total gene deletions, 23 multixon deletions and 3 multixon duplications. The subset analysis of *SPRED1* mutated group (n=19) showed 8 (42,5%) missense mutations, 6 (31,5%) frameshift, 2 (10,5%) nonsense, 2 (10,5%) splice errors and 1 (5%) total gene deletion. RNA analysis allowed us to identify 80/729 (11%) mutations that we would not be able to detect or characterize using DNA-based methodologies, as deep intronic mutations or other intronic changes out of canonical GT-AG splice sites. **Conclusions:** Our results confirm that RNA analysis together with MLPA provide a sensitive and specific method for the rapid identification of *NF1* and *SPRED1* mutations. RNA analysis improved our sensitivity over DNA-based methodologies, allowing accurate genetic counselling.

#### P12.161

#### Genetic diagnosis of NF1: A 5-year retrospective study evaluating different technologies

**M. Lazaro, M. Molero, L. Rausell, A. Gomez, A. Arteche, M. Bermejo, C. Ruiz, J. Garcia-Planells, M. Garcia-Hoyos;**  
*Instituto de Medicina Genómica (IMEGEN), Paterna, Spain.*

Neurofibromatosis type 1 (NF1; MIM#162200) is the most common autosomal-dominant neurocutaneous disorder associated with tumor predisposition. The primary clinical features of NF1 are multiple café-au-lait spots, axillary and inguinal freckling, Lisch nodules in the eye and benign peripheral nerve sheath tumors or neurofibromas. This disorder is caused by defects in the tumor suppressor gene NF1 (MIM\*613113). Mosaicism has been described to have important clinical implications in a large number of different diseases; therefore, it also plays an important role in some cases of NF1. During the last 5 years, we have analysed the NF1 gene in more than 150 patients using different techniques and approaches, including Sanger sequencing at mRNA level, NextGeneDX® (a post-PCR NGS methodology developed and validated by IMEGEN), and MLPA (Multiplex-Ligation Probe Amplification) at DNA level.

We have identified the disease-causing mutation in more than 60% of our studied cohorts and detected all types of truncated germlinal mutations: nonsense, splicing-site, and small and large deletions/duplications. Besides, NGS technologies enable the detection of disease-causing variations in mosaic. We describe the presence of mosaicism in different tissues and cell

lines in two cases. In one case, nonsense mosaicism disease-causing change (c.3916C>T; p.Arg1306\*) was detected. In the other case, two different changes in the same position of the cDNA, in at least two different cell lines (c.7806+2T>G and c.7806+2T>A), were detected. The combination of different techniques that includes NGS DNA sequencing, mRNA Sanger sequencing, and MLPA provides higher diagnostic yield for the detection of disease-causing mutations in NF1 patients.

#### P12.162

#### Comprehensive analysis of NF1 gene by using targeted resequencing and multiplex ligation-dependent probe amplification in patients with neurofibromatosis type 1

**S. Demir Ulusal<sup>1</sup>, H. Gürkan<sup>1</sup>, E. Atlı<sup>1</sup>, D. Eker<sup>1</sup>, S. A. Özal<sup>2</sup>, H. Tozkur<sup>1</sup>,**

<sup>1</sup>Trakya University Faculty of Medicine Department of Medical Genetics, Edirne, Turkey,

<sup>2</sup>Trakya University Faculty of Medicine Department of Ophthalmology, Edirne, Turkey.

**Introduction:**

NF1 gene consists 57 exons (NM\_000267.3) and involvement of various pathogenic variants including single nucleotide variants, in/dels and copy number variants makes difficult to analyse it. We aimed to summarize genetic analysis results of 17 neurofibromatosis patients studied between November 2014-January 2016.

**Methods:**

Genomic DNA was isolated from peripheral blood samples. NF1 coding regions were captured with IonAmpliseq DNA Library Preparation Kit and enriched by OT2 200 Kit. Resequencing performed on the Ion Personal Genome Machine. P081.B2 /P82.B2 and P373-B1 multiplex ligation dependent probe amplification (MLPA) probes were used to detect large deletions. ACMG 2015 guidelines, Human Genome Mutation Database, ClinVar, and Leiden Open Variation Database, were used for evaluation of the variant pathogenicity.

**Results:**

4 splice mutations, 2 frameshift deletions, 1 missense and 3 nonsense mutations defined. Additionally, NF1 whole gene deletion was found in a patient with MLPA. 27,2% of the mutations found were not previously reported.

**Conclusion:**

NF1 mutation frequency detected by genomic DNA sequencing was reported to be about 61% compared to our study (58,8%, Table1). MLPA increased the diagnostic yield up to 64,7%. There was at least one additional symptom to café-au-lait spots in most of the NF1 mutant patients (72,7%) compared to the patients without a mutation or deletion. We suggest that targeted resequencing and MLPA together offer a powerful diagnostic approach for neurofibromatosis in addition to clinical examination.

#### P12.163

#### The transfer of multigene panel testing for hereditary breast and ovarian cancer to healthcare: What are the implications for the management of patients and families?

**M. Eliade<sup>1</sup>, J. Skrzypski<sup>2</sup>, A. Baurand<sup>3</sup>, C. Jacquot<sup>4</sup>, G. Bertolone<sup>5</sup>, C. Loustalot<sup>6</sup>, C. Coutant<sup>3</sup>, F. Guy<sup>4</sup>, P. Fumoleau<sup>5</sup>, Y. Duffourd<sup>6</sup>, L. Arnould<sup>7</sup>, A. Delignette<sup>8</sup>, M. Padéano<sup>3</sup>, C. Lepage<sup>9</sup>, G. Raichon-Patru<sup>10</sup>, A. Boudrat<sup>11</sup>, M. Bône-Lépinoy<sup>12</sup>, A. Villing<sup>13</sup>, S. Chevrier<sup>2</sup>, F. Végran<sup>7</sup>, N. Sevenet<sup>14</sup>, F. Ghiringhelli<sup>15</sup>, R. Boidot<sup>7</sup>, S. Lizard<sup>7</sup>, L. Faivre<sup>1</sup>,**

<sup>1</sup>Centre de Génétique, Hôpital des Enfants, Dijon, France, <sup>2</sup>Unité d'oncogénétique, Centre Georges-François Leclerc, Dijon, France, <sup>3</sup>Service de Chirurgie, Centre Georges-François Leclerc, Dijon, France, <sup>4</sup>Service de Radiologie, Centre Georges-François Leclerc, Dijon, France, <sup>5</sup>Oncologie Médicale, Centre Georges-François Leclerc, Dijon, France, <sup>6</sup>Orphanomix, Dijon, France, <sup>7</sup>Service de Biologie et pathologie des tumeurs, Centre Georges-François Leclerc, Dijon, France, <sup>8</sup>Tour Elithis, Radiologie, Dijon, France, <sup>9</sup>Service de Gastroentérologie, Hôpital François Mitterrand, CHU, Dijon, France, <sup>10</sup>Service d'Oncologie, Centre Hospitalier Les Chanaux, Mâcon, France, <sup>11</sup>Service d'Oncologie, Centre Hospitalier William Morey, Chalon-sur-Saône, France, <sup>12</sup>Service d'Oncologie-Radiothérapie, Clinique Drevon, Dijon, France, <sup>13</sup>Service d'oncologie, Centre Hospitalier, Auxerre, France, <sup>14</sup>Laboratoire de génétique moléculaire Département de bio-pathologie, Institut Bergonié, Bordeaux, France.

Until recently, the molecular diagnosis of hereditary breast and ovarian cancer (HBOC) was mostly based on BRCA1/2 testing. Next generation sequencing and the recent discovery of new genes involved in HBOC progressively permitted the transfer of genomic capture targeting multiple candidate genes from research to diagnosis. However, the implication for the management of patients and their families has not been extensively studied. We studied 583 consecutive patients originated from Burgundy (France) fulfilling the criteria for BRCA testing using a next generation sequencing 25-gene panel including 20 candidate predisposition genes for breast and/or ovarian cancer. A pathogenic BRCA1/2 mutation was found in 51 patients (9%). Besides, we found 37 pathogenic or likely pathogenic mutations in 10 different high to low-risk genes in 34 patients (6%). The most fre-

quently mutated genes were CHEK2 (n=12; 2 %), ATM (n=9; 1.5 %), and PALB2 (n=4; 0.6%). Three double-hits and four mutations in MMR genes were also found. Most of mutations were consistent with the spectrum of cancers observed in the patient and/or family (89%). The analysis of clinical actionability performed among the mutation-positive individuals other than BRCA revealed that additional disease specific screening and/or prevention measurers beyond those based on personal and family history alone have been recommended in most of cases (84%). In conclusion, multigene panel testing is a powerful tool for identifying high to low-risk HBOC susceptibility genes. The penetrance and spectrum of cancer associated to these other genes remain sometimes undefined, and further collaborative work is needed to address this question.

#### P12.164

#### Targeted next generation sequencing identifies a novel mutation in FANCA gene

*M. Dimishkovska<sup>1</sup>, S. A. Kocheva<sup>2</sup>, D. Plaseska-Karanfilska<sup>1</sup>;*

*<sup>1</sup>Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>Pediatric Clinic, Medical Faculty, Skopje, Macedonia, The Former Yugoslav Republic of*

Fanconi anemia (FA) is a rare autosomal recessive or X-linked disorder characterized by clinical and genetic heterogeneity. Characteristic clinical features include developmental abnormalities in major organ systems, bone marrow failure and genomic instability resulting to cancer predisposition. Analysis of three unrelated gipsy families in Macedonia had reviled a homozygous deletion of exon 3 in FANCA gene (c.190-256\_283+1680del2040dupC) as a founder mutation in Macedonian FA patients of Gypsy-like ethnic origin (Acta Hematol 2014;132:15-21).

Case report: We report a girl from gipsy minority who was referred to the pediatric clinic at the age of 2,5. The patient had normochromic anemia with thrombocytopenia, increased level of fetal hemoglobin (10.4%) and no skeletal or organ systems abnormalities. After a few months the disease progressed and the intracranial hemorrhage resulted in death.

Due to suspicion of FA, we analyzed the proband for the known founder mutation (deletion c.190-256\_283+1680del2040dupC) but we detected the deletion in a heterozygous state. In order to clarify the diagnosis, a targeted resequencing was performed using an Illumina kit TruSight One sequencing panel.

Our filtering approach revealed an undescribed heterozygous mutation c.3446\_3449dupCCCT (p.Met1151ProfsTer65) in FANCA gene, located in the region of interaction with the protein FAAP20 important for the functional integrity of the FA pathway.

In conclusion, using targeted NGS we detected a novel mutation in FANCA gene in a patient with Fanconi anemia from Macedonia with gipsy ethnic origin.

#### P12.165

#### Multiple gene panel testing unravels rare aspects of cancer genetics

*F. Fostira, I. Konstantopoulou, P. Apostolou, I. Konstanta, V. Mollaki, A. Vagena, M.*

*Papamantzelopoulou, D. Yannoukakos;*

*Molecular Diagnostics Laboratory, INRASTES, NCSR, Demokritos, Athens, Greece.*

Next-generation sequencing (NGS) and more specifically, customized gene panels, have replaced conventional Sanger sequencing and became the gold standard for hereditary cancer diagnostics. Through this technology, we report a number of interesting findings, which would have been missed by other methods.

Case 1. A female with early onset breast cancer was found to be a double heterozygote for loss-of-function mutations in both PTEN and PALB2 genes. Case 2. A rare case of TP53 mosaic event has been detected in a man who was previously diagnosed with four primary cancers. The TP53 missense mutation, located in the DNA binding domain, has been identified in 9.2% of the total reads.

Case 3. Family relatives within a family with multiple cases of ovarian cancer were found to carry two different BRCA1 deleterious mutations.

Case 4. Two sisters diagnosed with early onset breast cancer. One of them carries a PALB2 missense mutation with potential pathogenicity, while the other carries a BRCA2 pathogenic mutation.

Case 5. In a family with three cases of premenopausal breast cancer, proband tested negative, while a MEN1 pathogenic mutation was detected in the second member tested. Subsequently, her daughter was diagnosed with neuroendocrine pancreatic cancer and was also found to carry the MEN1 mutation.

All the above highlight the different aspects in genetics that arise through the evolution of NGS technologies, from explaining missing heritability and

elucidating unclear phenotypes, to raising new questions about whether to report incidental findings and whether predictive testing versus full testing is always sufficient.

#### P12.166

#### Molecular diagnosis of inherited colorectal cancer using NGS panel

*S. Baert-Desurmont, F. Charbonnier, S. Coutant, M. Vezain, R. Lanos, J. Bou, E. Bouvignies, S. Fourneau, S. Manase, S. Vasseur, G. Bougeard, J. Mauillon, I. Tournier, T. Frebourg;*

*Department of Genetics Rouen University Hospital and Inserm U1079, Rouen University, Normandy Centre for Genomics and Personalized Medicine, Rouen, France.*

We have developed and optimized a massive parallel sequencing strategy for the diagnosis of inherited forms of colorectal cancer (CRC). This strategy is based on (1) a panel of 10 genes involved in Mendelian forms of CRC (MLH1, MSH2, MSH6, PMS2, APC, MUTYH, STK11, SMAD4, BMPR1, PTEN), (2) a quick capture of exonic and intronic sequences using Sureselect Agilent QXT, (3) sequencing on MiSeq and NextSeq 500 Illumina platforms, (4) double bioinformatics pipelines including CASAVA (Illumina) and BWA-GATK (Broad Institute) softwares for alignment and variant calling, Alnmut Batch (Interactive BioSoftware) for annotation, completed by CANOE software for the rearrangement detection, (5) automatically generated quality reports and (6) systematic control of the genotypes, using Sanger sequencing or QMPSF for the positive cases and Multiplex SnaPshot analysis of SNPs for the negative cases. Analysis of 1200 index cases allowed us to identify a deleterious mutation in 18% of the patients and the mutation detection rate reached 32% in Lynch syndrome suspected by tumour analyses. The main advantages of this strategy are the reduction of molecular diagnosis delay, the correction of the diagnosis in cases of overlapping phenotypes (MUTYH biallelic mutation mimicking Lynch syndrome) and the detection of mosaics and cryptic alterations.

#### P12.167

#### Comprehensive identification of genes driven by ERV9-LTRs reveals TNFRSF10B as a re-activatable mediator of testicular cancer cell death

*U. Beyer<sup>1,2</sup>, S. K. Kroenung<sup>1</sup>, A. Leh<sup>3</sup>, L. Walter<sup>4</sup>, M. Dobbelstein<sup>1</sup>;*

*<sup>1</sup>University Goettingen, GZMB, Department of Molecular Oncology, Goettingen, Germany,*

*<sup>2</sup>Medical University Hannover, Department of Human Genetics, Hannover, Germany,*

*<sup>3</sup>University Goettingen, Department of Medical Statistics, Goettingen, Germany, <sup>4</sup>German Primate Center, Primate Genetics Laboratory, Goettingen, Germany.*

The long terminal repeat (LTR) of human endogenous retrovirus type 9 (ERV9) acts as a germline-specific promoter that induces the expression of a proapoptotic isoform of the tumor suppressor homologue p63, GTAp63, in male germline cells. Testicular cancer cells silence this promoter, but inhibitors of histone deacetylases (HDACs) restore GTAp63 expression and give rise to apoptosis. We show here that numerous additional transcripts throughout the genome are driven by related ERV9-LTRs. 3' Rapid amplification of cDNA ends (3' RACE) was combined with next-generation sequencing to establish a large set of such mRNAs. HDAC inhibitors induce these ERV9-LTR-driven genes but not the LTRs from other ERVs. In particular, a transcript encoding the death receptor DR5 originates from an ERV9-LTR inserted upstream of the protein coding regions of the TNFRSF10B gene, and it shows an expression pattern similar to GTAp63. When treating testicular cancer cells with HDAC inhibitors as well as the death ligand TNF-related apoptosis-inducing ligand (TRAIL), rapid cell death was observed, which depended on TNFRSF10B expression. HDAC inhibitors also cooperate with cisplatin (cDDP) to promote apoptosis in testicular cancer cells. ERV9-LTRs not only drive a large set of human transcripts, but a subset of them acts in a proapoptotic manner. We propose that this avoids the survival of damaged germ cells. HDAC inhibition represents a strategy of restoring the expression of a class of ERV9-LTR-mediated genes in testicular cancer cells, thereby re-enabling tumor suppression.

#### P12.168

#### Germline mutations in 94 cancer predisposition genes among large epithelial ovarian cancer cohort

*R. Janavičius<sup>1,2</sup>, V. Rudaitis<sup>3</sup>, N. Archipova<sup>1</sup>, L. Griškevičius<sup>1</sup>;*

*<sup>1</sup>Vilnius University Hospital Santariskiu Clinics Hematology, Oncology and Transfusion Medicine Center, Vilnius, Lithuania, <sup>2</sup>State Research Institute Innovative Medicine Center, Vilnius, Lithuania, <sup>3</sup>Vilnius University Hospital Santariskiu Clinics, Dept. of Oncogynecology, Vilnius, Lithuania.*

INTRODUCTION. Recent advances in massive parallel DNA sequencing led to the generation and application of various cancer predisposition genes (CPG) panels, improving the stratification of genetic cancer subtypes for better

management and targeted therapy. To determine the utility of multigene testing approach we assessed the frequency of pathogenic variants in 94 pan-CPGs in a large epithelial ovarian cancer (EOC) cohort from a homogenous population of Lithuania.

**METHODS.** Patients with EOC (n=586) w/wo family history (FH) were recruited through single hospital center (VUHSK HOTC) and germline DNA was sequenced and analysed using Illumina MiSeq multigene panels of 94 CPGs, including BRCA1 and BRCA2.

**RESULTS.** Deleterious (class IV/V) mutations were identified in 35.6% all EOC patients. Of these, 29.4% had mutations in BRCA1 (26.5%) and BRCA2 (2.9%) genes, whereas 6.3% harboured mutations in other CPG, including RAD51C, BRIP1, ATM, STK11, MUTYH, MLH1, MSH6, TP53, CHEK2, WRN, FANC. In patients w/o FH pathogenic BRCA1/2 mutations were found in 18.3% and 4.2% harboured mutations in other genes. EOC patients with pathogenic CPG mutations were diagnosed at an earlier age (p<0.001).

**CONCLUSION.** The heritable component of EOC due to mutations in other genes than BRCA1/2 genes is less prevalent and not affected by FH. Clinical utility for newer genes remains to be established.

#### P12.169

#### Analysis of germline HABP2 G534E variant in Spanish families with Nonmedullary Thyroid Cancer

*M. Ruiz-Ferrer<sup>1,2</sup>, R. M. Fernández<sup>1,2</sup>, E. Navarro<sup>3,2</sup>, G. Antíñolo<sup>1,2</sup>, S. Borrego<sup>1,2</sup>;*

<sup>1</sup>Department of Genetics, Reproduction and Fetal Medicine, Institute of Biomedicine of Seville (IBIS), University Hospital Virgen del Rocío/CSIC/University of Seville, Seville, Spain, <sup>2</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Seville, Spain, <sup>3</sup>Department of Endocrinology, Institute of Biomedicine of Seville (IBIS), University Hospital Virgen del Rocío/CSIC/University of Seville, Seville, Spain.

Nonmedullary thyroid cancer (NMTC) comprises thyroid cancers of follicular cell origin and accounts for more than 95% of all thyroid cancer cases. Recently, using NGS technologies and further functional studies, the G534E variant in *HABP2* gene has been implicated in familial NMTC. However, the role of this variant has generated the latter scientific comments due to the higher reported frequencies in public database. Subsequently, several cohorts of patients from different ethnical origin have been evaluated with different results reported. Therefore, the added collaborative effort of testing this variant in NMTC families has been requested to further clarify its role. In this sense, we have evaluated the G534E variant in 14 Spanish families with NMTC (13 papillary thyroid cancer, PTC; and 1 follicular thyroid cancer, FTC), including 30 affected and 46 unaffected individuals. As a result, we only found two heterozygous variant carriers, both in the same family, but clearly showing no segregation between PTC and the G534E variant. Specifically, there was one PTC patient in this family who does not carry the *HABP2* variant and one of the identified carrier was an unaffected individual. In addition, we examined this variant in 267 Spanish controls exomes available in the context of the Medical Genome Project. Among them, 6 individuals (2.24%) were heterozygous for the variant (MAF = 0.0112). According to this, our results suggest that the G534E variant in *HABP2* might not be associated with familial NMTC in the Spanish population.

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#### P12.170

#### Oral squamous cell carcinoma: can the genes predict a second primary tumor?

*I. M. Carreira<sup>1,2</sup>, I. P. Ribeiro<sup>1,2</sup>, F. Marques<sup>2,3,4</sup>, F. Caramelo<sup>5</sup>, A. Santos<sup>1</sup>, M. J. Julião<sup>6</sup>, I. P. Baptista<sup>2,3</sup>, J. B. Melo<sup>1,2</sup>;*

<sup>1</sup>Cytogenetics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal,

<sup>2</sup>CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, Coimbra, Portugal, <sup>3</sup>Department of Dentistry, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>4</sup>Stomatology Unit, Coimbra Hospital and University Centre, CHUC, EPE, Coimbra, Austria, <sup>5</sup>Laboratory of Biostatistics and Medical Informatics, IBILI - Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>6</sup>Department of Pathology, Coimbra Hospital and University Centre, CHUC, EPE, Coimbra, Portugal.

**Introduction:** Oral squamous cell carcinoma (OSCC) patients are at elevated risk of second primary tumors (SPT) with significant reduction of 5-year survival. For OSCC management the identification of molecular markers with predictive value of relapse risk development and of response to the treatment is pivotal. **Material and Methods:** We identified the genomic and epigenetic profile of a primary tongue tumor and of a SPT in the floor of the mouth of the same patient, diagnosed four years and eight months later. Primary tumor, SPT and macroscopically tumor free tissue from SPT were analyzed. This study was conducted using array-Comparative Genomic Hybridization and Methylation-Specific Multiplex Ligation-dependent Probe

Amplification. **Results:** The patient was 49 years old male at the time of the primary tumor diagnosis and a heavy smoker (>20 cigarettes/day). Treatment of primary tumor was radiochemotherapy and surgery for SPT. SPT presented more aberrations than primary tumor. However, primary tumor and SPT shared several genomic imbalances, namely at 17 and 19 chromosomes. Copy number alterations in genes related with radioresistance and worse prognosis were identified. WT1, CHFR and GATA5 methylated in tumor and macroscopically tumor free tissue of SPT were detected. Three months after SPT diagnosis the patient presented a tumor in larynx. **Conclusions:** Genomic and epigenetic profile of tumors in different anatomic subsites seems to be different, even in the same patient exposed to the same risk factors. The molecular signatures seem to be pivotal to help in the early detection of SPT, to predict tumor behavior and response to therapy.

#### P12.171

#### Ovarian carcinomas and hereditary cancer syndromes

*L. Libera<sup>1</sup>, I. W. Carnevali<sup>2</sup>, L. Cimetti<sup>2</sup>, N. Sahnane<sup>1</sup>, T. Rossi<sup>3</sup>, G. Formenti<sup>3</sup>, A. Chiaravall<sup>2</sup>, D. Furlan<sup>1</sup>, C. Riva<sup>1</sup>, M. G. Tibiletti<sup>2</sup>;*

<sup>1</sup>University of Insubria, Varese, Italy, <sup>2</sup>Ospedale di Circolo, Varese, Italy, <sup>3</sup>Ospedale del Ponte, Varese, Italy.

**Introduction:** About 23% of ovarian carcinomas (OC) are related to hereditary conditions, mainly Hereditary Breast Ovarian Cancer (HBOC) and Lynch syndrome (LS). The identification of germline mutation represents a fundamental step in the management of these patients and their relatives. **Patients:** 126 women affected by OC referred to our Genetic Counseling Service for a suspected inherited cancer syndrome. 110/126 patients underwent BRCA1/2 or MMR germline genetic test based on their family history and/or somatic analyses for MMR defects.

**Results:** Somatic MMR analysis was performed on 45 OC and 14 of these resulted MMR defective. Six of 14 patients revealed MMR pathogenetic germline variants, 5 showed variants of uncertain significance (VUS) and 3 had sporadic cancers. In the remaining 96 patients with OC, the germline BRCA test was performed and a pathogenetic variant of BRCA1 or BRCA2 genes was identified in 43 women. The global detection rate (DR) of hereditary syndromes was 45% (49/110). Mutated and wildtype patients had the same age of OC onset (50.2ys). Interestingly, the mean age of OC onset in LS patients was lower than that of HBOC patients (45ys vs 51.6ys). Regard to histological types of OC, in both LS and HBOC patients, endometrioid, serous, clear cells and mixed carcinomas and carcinosarcomas were observed suggesting that in these syndromes is not involved an exclusive OC histological type.

**Conclusions:** The OC is a sentinel cancer for inherited cancer syndromes identification independently to histological type and age of onset.

#### P12.172

#### A Hot-spot of In-frame Duplications Activates the Oncoprotein AKT1 in Juvenile Granulosa Cell Tumors

*A. Todeschini<sup>1</sup>, L. Bessière<sup>1</sup>, A. Auguste<sup>1</sup>, S. Sarnacki<sup>2</sup>, D. Flatters<sup>3</sup>, B. Legois<sup>1</sup>, A. Zider<sup>1</sup>, S. Caburet<sup>1</sup>, C. Sultan<sup>4</sup>, N. Kalfa<sup>4</sup>, L. Galmiche<sup>2</sup>, R. A. Veitia<sup>1</sup>,*

<sup>1</sup>Institut Jacques Monod/CNRS/Université Paris Diderot-Paris VII, PARIS, France,

<sup>2</sup>Hôpital Necker Enfants-Malades/Université Paris Descartes-Paris V, PARIS, France,

<sup>3</sup>INSERM UMR973/Université Paris Diderot-Paris VII, PARIS, France, <sup>4</sup>University Hospital of Montpellier/Hôpital Lapeyronie, MONTPELLIER, France.

Ovarian granulosa cell tumors are the most common sex-cord stromal tumors and have juvenile (JGCTs) and adult forms. The molecular basis of JGCTs is poorly understood although mutations in the GNAS gene have been reported but only in 30% of the analyzed JGCTs. We have searched for alterations in other proteins involved in ovarian mitogenic signaling. This led us to detect in-frame duplications within the oncogene AKT1 in >60% of the JGCTs studied and point mutations affecting highly conserved residues in tumors without duplications. Subcellular localization analyses of the mutated proteins and functional explorations using Western-blot and luciferase assays showed that AKT1 variants bearing the duplications located at the plasma membrane and were hyperactive. To gain insights into the effects of these mutations and the existence of potential co-driver alterations, we performed transcriptome of four JGCTs. RNA-Seq data analyses showed that the duplications were the sole lesions common to the four tumors suggesting a causative role. They also pinpointed a series of differentially expressed genes, involved in cytokine and hormone signaling and cell division-related processes. Our results suggested that most of the transcriptomic dysregulation might be mediated by a limited set of transcription factors perturbed by AKT1 activation. Finally, we show that commercially available AKT inhibitors can modulate the in vitro activity of various mutated forms. Our study incriminates somatic AKT1 mutations as a major event in the pathogenesis of JGCTs and provide therapeutic leads for a targeted treatment.

## P12.173

### Exploring the non-randomness of somatic mutations in cancer genomes

**M. D. Stobbe<sup>1,2</sup>, D. Kedra<sup>1,2</sup>, M. Oliva<sup>1,2,3</sup>, E. Rainieri<sup>1,2</sup>, I. G. Gut<sup>1,2</sup>, Pan-Cancer Analysis of Whole Genomes study of the International Cancer Genome Consortium;**  
<sup>1</sup>CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>University of Chicago Department of Medicine & Institute for Genomics and Systems Biology, Chicago, IL, United States.

Mutation rates in tumors are higher than basal mutation rates in unaffected tissues. However, given the sheer size of the human genome it is still improbable that identical mutations are recurrent by chance. To explore this hypothesized non-randomness of somatic mutations we used a pilot dataset of 2049 cancer genomes from the Pan-Cancer Analysis of Whole Genomes project of the International Cancer Genome Consortium, for which the somatic mutation calls were available from one of the pipelines used in this project. We first normalized the mutation calls to make them comparable across samples and applied the filters provided by the pipeline. To reduce false positives, we removed all calls found in Kaviar or dbSNP, two variant databases. This left us with ~31 million unique Somatic Single-base Mutations (SSMs) of which ~43.000 are recurrent, *i.e.* present in 3 or more tumours, and ~2 million unique Somatic Insertion/deletion Mutations (SIMs) of which ~130.000 are recurrent. We selected the top 10 ranking recurrent SSMs, SIMs and 2bp deletions, which define 30 sets of samples, to identify genomic and phenotypic events that co-occur with the selected recurrent mutation.

Multiple sets of samples share, *i.a.*, more mutations between them than randomly selected samples without the specific mutation. Nearly all sets contain samples from different cancer types suggesting relevant pan-cancer commonalities. In one such case with samples from colon, stomach and uterine cancer, the processes behind the non-randomness of the mutations may be the DNA damage repair pathways that are often defective in these three cancers.

## P12.174

### The pancreatic master genes expression in Panc1 cells stimulated with TGFβ1

**L. Kondratyeva<sup>1</sup>, A. Sveshnikova<sup>1,2</sup>, I. Chernov<sup>1</sup>, E. Kopancev<sup>1</sup>, E. Sverdlov<sup>1,3</sup>;**  
<sup>1</sup>M.M. Shemyakin and Yu.A. Ovchinnikov Institute of bioorganic chemistry of the Russian Academy of Science, Moscow, Russian Federation, <sup>2</sup>Lomonosov Moscow State University, Moscow, Russian Federation, <sup>3</sup>Institute of Molecular Genetics Russian Academy of Sciences, Moscow, Russian Federation.

Introduction: One of the most important events of cancer progression is an epithelial-mesenchymal transition (EMT). Activation of EMT program is realized by mean growth factors, for instance, TGFβ1. The goal of this project was expression level analysis of genes of master factors that manage developing pancreas in cell line Panc1 stimulated with TGFβ1

Materials and Methods: To perform this study we cultivated Panc1 cells during 120 hours with TGFβ1. Changes in the cells were analyzed by Western blot test and qRT-PCR for the 4 genes marked EMT and the 13 master factors genes.

Result: Stimulation of Panc1 with TGFβ1 has resulted in changing cell epithelial to mesenchymal morphology, increasing level of SNAIL and decreasing level of E-cadherin. Besides the relative expression levels of SNAI1 and SNAI2 genes increased 6- and 25-fold, respectively, expression levels of CDH1 and KRT8 decreased 25- and 15-fold, respectively. For the selected master genes suppression of expression level SOX9 twice, FOXA2 5-fold and GATA4 4-fold was revealed. Expression of PTF1A, PDX1, HNF1b, NEUROG3 genes was not detected, expression level of GATA6, HES1, NKX6.1, NR5A2, RBPJL and ONECUT1 has not changed.

Conclusion: Visual observation during 120 hours and changing profiles of protein and genes marked EMT after incubation with factor prove that addition of TGFβ1 in the cell medium induce EMT process. Among the 13 selected genes of master factor of pancreas development SOX9, FOXA2 and GATA4 genes changed their expression levels.

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## P12.175

### Regulation of GAD expression in human pancreatic cancer cell lines

**M. L. Svrtlih<sup>1,2</sup>, D. Drakulic<sup>1</sup>, Z. Katarova<sup>2</sup>, M. Mojsin<sup>1</sup>, Z. Mate<sup>2</sup>, M. Stevanovic<sup>1</sup>, G. Szabo<sup>2</sup>;**  
<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Division of Medical Gene Technology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

Introduction: Pancreatic ductal adenocarcinoma (PDAC), most common

type of pancreatic cancer is an aggressive disease with dismal prognosis mainly because it is generally discovered very late and it is resistant to chemotherapy. Aside from the role as a major inhibitor neurotransmitter in the brain, GABA ( $\gamma$ -aminobutyric acid) has emerged as a stem and cancer cell signaling molecule in different tissues. Glutamic acid decarboxylase (GAD), the biosynthetic enzyme for GABA, exists in two forms- GAD65 and GAD67 that are usually co-expressed in various ratios in the GABAergic cells but differ in subcellular localization, kinetics and cofactor binding.

Materials and Methods: The RT-PCR and immunocytochemistry assays were used to access the gene and protein expression in three PDAC cell lines, Panc1, MiaPaCa2 and HPAF II.

Results: Our results show that, in contrast to healthy exocrine pancreas PDAC cell lines express the GAD isoforms, GABA receptors and accumulate GABA. Specific activation of GABA receptors significantly increased the proliferation rate of these cells. Finally, following the treatment with the chemotherapeutic agent retinoic acid we detected altered expression of GAD65/GAD67 genes, as well as cancer stem cell transcription factor, SOX2.

Conclusion: The identification of molecules and proteins involved in development and progression of PDAC is critical to develop novel active drugs. Our data provide new insights into the role of GABA in pancreas as a modulator of cellular proliferation and/or differentiation during tumorigenic cell transformations. This work was partially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No. 173051)

## P12.176

### Expression of survivin and its splice variants in pediatric acute lymphoblastic leukemia

**E. Eren-Keles<sup>1</sup>, H. G. Karabulut<sup>2</sup>, H. F. Cakmakli<sup>3</sup>, B. Adakli-Aksoy<sup>3</sup>, S. K. Kose<sup>4</sup>, H. Dincaslan<sup>2</sup>, L. G. Yavuz<sup>2</sup>, M. Ertem<sup>2</sup>, A. Tukun<sup>2</sup>;**

<sup>1</sup>Ankara University Institute of Biotechnology, Ankara, Turkey, <sup>2</sup>Ankara University School of Medicine, Department of Medical Genetics, Ankara, Turkey, <sup>3</sup>Ankara University School of Medicine, Department of Pediatric Hematology and Oncology, Ankara, Turkey, <sup>4</sup>Ankara University School of Medicine, Department of Biostatistics, Ankara, Turkey.

Introduction: Survivin (BIRC5) is a member of the inhibitor of apoptosis protein family and involved in inhibition of apoptosis and regulation of cell division. High expression of survivin mRNA has been shown in several human cancers including hematological malignancies. However, expression level of survivin in pediatric acute lymphoblastic leukemia (ALL) and its prognostic value has not been well studied.

Materials and Methods: 41 pediatric B-precursor ALL patients and six healthy donors were included in this study. Expression of survivin and its splice variants including  $\Delta$ Ex3, 2B, 3B and 2 $\alpha$  was investigated by real-time PCR both at first diagnosis and in remission periods of patients divided into high (n=16) and standard risk (n=25) groups according to age, white blood cell count and genetic risk factors. In addition, survivin variant(s) that may affect the response to the treatment was also investigated.

Results: Expression levels of survivin and survivin- $\Delta$ Ex3 were found to be higher in patient group than controls, and survivin-3B was found at increased levels in high risk group both at first diagnosis and remission periods. The ratio of survivin-2 $\alpha$ /3B expression was also higher at diagnosis than remission period.

Conclusion: Our results suggest that survivin and survivin- $\Delta$ Ex3 may be a risk factor for childhood ALL. On the other hand, survivin-3B can be used in risk classification and as a therapeutic target. Survivin-2 $\alpha$ /3B and survivin- $\Delta$ Ex3/WT may also be taken into consideration in risk classification.

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## P12.177

### Microdeletion 19p13.3 including STK11 gene in a patient with Peutz-Jeghers syndrome and dysmorphic features

**C. Machado, P. Dias, J. Dupont, R. Silveira-Santos, S. Serafim, A. Sousa;**  
 Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Introduction: Peutz-Jeghers (PJS) is an autosomal dominant cancer predisposition syndrome characterized by gastrointestinal hamartomatous polyposis and mucocutaneous pigmentation. Germline point mutations and partial/whole exon deletions in STK11 can be identified in about 70% and 30% of patients with PJS, respectively. Only a few carriers of large genomic deletions at 19p13.3 encompassing STK11 have been previously reported. Case presentation: A 31 year-old woman was referred for genetic evaluation for dysmorphism. Family history was unremarkable. She had normal intel-

lect. In her late teens, a jejunal villous polyp and pseudopolyps of the ileus and cecum had been removed. She had also been noticed to have lip pigmentation. However, sequencing of STK11 found no mutation. On observation, she presented thick eyebrows, long down-slanting palpebral fissures, ocular proptosis, high nasal bridge, hanging columella, black-pigmented spots on the lips and a sandal gap.

Results: MLPA using DNA extracted from peripheral blood revealed a probable total deletion of one copy of STK11: c. (?\_1100)\_(?3276\_?); array-CGH (NimbleGen CGX 135K, Perkin Elmer) confirmed the presence of a clinically significant interstitial deletion (410.30kb) within 19p13.3. The deletion was de novo and involved 13 OMIM genes, including STK11.

Conclusion: PJS features in our patient are a consequence of STK11 haploinsufficiency. Dysmorphisms common to our patient and similar reported cases in the literature support the notion that genes in the neighborhood of STK11 are responsible for a clinically recognizable phenotype. This case strengthens the need to screen for large deletions in patients presenting with PJS together with dysmorphic and/or other atypical clinical features.

#### P12.178

#### Whole-genome microarray analysis to establish genetic profiles of pilocytic astrocytomas

J. N. Sanmann<sup>1</sup>, A. T. Calleroz<sup>2</sup>, J. M. Stevens<sup>3</sup>, M. Nelson<sup>1</sup>, P. A. Althof<sup>4</sup>, D. L. Pickering<sup>1</sup>, N. Lu<sup>1</sup>, C. Lettieri<sup>3</sup>, L. J. Starr<sup>4</sup>, D. Perry<sup>5</sup>;

<sup>1</sup>Human Genetics Laboratory, Munroe-Meyer Institute at the University of Nebraska Medical Center, Omaha, NE, United States, <sup>2</sup>Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States, <sup>3</sup>Department of Pediatrics Hematology/Oncology, University of Nebraska Medical Center, Omaha, NE, United States, <sup>4</sup>Department of Genetic Medicine, Munroe-Meyer Institute at the University of Nebraska Medical Center, Omaha, NE, United States, <sup>5</sup>Department of Pathology, Children's Hospital & Medical Center, Omaha, NE, United States.

Introduction: Pilocytic astrocytomas, the most common pediatric brain tumor, are low-grade gliomas. Although distinct clinical features exist, the histopathological diagnosis of pilocytic astrocytoma can be challenging because of the wide morphologic spectrum and histopathological overlap with high grade malignancies. A MAPK pathway aberration involving BRAF has been reported in pilocytic astrocytomas, but nearly one-third lack the presence of a unique, detectable genetic hallmark. This study aimed to molecularly characterize a cohort of pilocytic astrocytomas for identification of additional genetic signatures that may provide additional information to assist in making the correct pathologic diagnosis.

Methods: DNA was extracted from archived paraffin-embedded tissue from 18 pediatric pilocytic astrocytomas. Microarray was performed using the Affymetrix OncoScan® FFPE Assay according to standard protocol (Santa Clara, CA) for identification of clinically-relevant copy number variants (CNVs) and regions of copy-neutral loss of heterozygosity (CN-LOH).

Results: CNVs involving BRAF were identified in 13/18 (72%) of cases: gain encompassing both KIAA1549 and BRAF loci (n=12) and deletion in BRAF (n=1). Whole chromosome gains were the next frequent CNV noted, with recurrent involvement of chromosomes 5 and 6 (n=2). Additional, less frequent CNVs and CN-LOH were present throughout the cohort.

Conclusions: Microarray analysis is a cost-effective tool for the identification of the hallmark genetic aberrations reported in pilocytic astrocytomas, including deletions and duplications of BRAF. Additionally, the whole-genome platform allows for characterization of additional neoplastic markers that may have diagnostic and prognostic implications.

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#### P12.179

#### Analytical challenges in the study of PMS2 for Lynch syndrome genetic diagnosis. Long range PCR matters

V. M. Barberá, M. I. Castillejo, A. Castillejo, J. L. Soto;  
Hospital Universitario Elche, Elche, Spain.

Introduction: Genetic tests for Lynch Syndrome are well standardized and broadly used, although there remain some specific difficulties that need to be addressed to reach an optimal diagnosis. The analysis of PMS2 gene is especially complex because of the high number and homology with several pseudogenes. We report the problem raised by differences in sensibility and specificity to detect genetic variants depending on polymerases enzyme blends and master mixes used for long range PCRs (LR-PCR).

Materials and Methods: Two cases with suspicious of Lynch Syndrome were tested for mutations in PMS2 using two different commercial LR-PCR kits in parallel (LongRange\_PCR\_kit, QIAGEN and Expand\_LonRange\_dNTPack, Roche). The amplicons generated by LR-PCR contained the exons 1-5 (9.964bp), exons 11-12 (8812bp) and exons 13-15 (9804bp). Next, we performed nested PCR by the same master mix (AmpliTaq Gold 360, Life Tech-

nologies).

Results: Using the first LR-PCR kit we detected a new pathogenic variant: 97bp deletion in I01-E02 boundary (c.24-12\_109del) that was confirmed in independent experiments and both strands. This mutation could not be detected using the second LR-PCR kit. Moreover, the analysis of a second case showed a missense variant in E02:c.137G>T;p.(Ser461le) detected by the second, but not with the first LR-PCR kit. This variant could not be confirmed in both strands and was considered as an artifact.

Conclusions: LR-PCR can be used to amplify the PMS2 gene and avoid pseudogene interference previously to sequence (Sanger or NGS) although a detailed validation analysis is needed to provide a clinically useful analysis of PMS2.

#### P12.180

#### Germline POLD1 mutation can mimic Lynch syndrome

M. Castillejo<sup>1</sup>, A. Castillejo<sup>1</sup>, E. Hernández-Illán<sup>1</sup>, I. Tena<sup>2</sup>, J. Soto<sup>1</sup>;

<sup>1</sup>Hospital Universitario Elche, Elche, Spain, <sup>2</sup>Hospital Provincial Castellón, Castellón, Spain.

Introduction: Lynch syndrome (LS) is caused by pathogenic germline variants in one of the mismatch repair (MMR) genes and patients have a high risk of developing colorectal cancer and other tumors. Microsatellite instability (MSI) and loss of MMR protein expression are the molecular hallmarks of LS tumors. Recently, it has been suggested that mutations in POLE and POLD1 genes might explain part of the suspected LS cases without mutations in MMR genes.

Materials/Methods: We analyzed germline mutations at POLE (exon 13) and POLD1 (exon 11) genes in 327 unexplained suspected LS cases. From these, 158 tumors had normal expression and 165 tumors had loss of expression of MMR proteins. Analysis of MLH1 somatic mutations in tumor was performed in one case of interest.

Results: We found three mutated individuals among cases with normal MMR protein expression (1.9%). Two cases had POLD1\_Leu474Pro mutation and one case had POLE\_Leu424Val mutation. These three cases fulfilled Amsterdam II criteria.

Another individual with the POLD1\_Leu474Pro mutation was found among cases with loss of MMR protein expression (0.6%). This patient was diagnosed of colorectal cancer at age 51 with no family history of cancer. His tumor showed loss of MLH1 expression without MLH1 promoter methylation and wild-type for BRAF\_V600E mutation. Germline analysis of MLH1 did not show any pathogenic alteration. Tumor analysis of MLH1 showed a homozygous/hemizygous nonsense pathogenic variant [(c.1279C>T; p.(Glu427\*)] suggesting somatic inactivation of MLH1 as a consequence of POLD1 deficiency.

Conclusion: Mutations at POLD1 can induce tumorigenesis via MSI-pathway mimicking Lynch syndrome.

#### P12.181

#### Combined mismatch repair and POLE/POLD1 defects explain unresolved suspected Lynch Syndrome cancers

A. M. L. Jansen<sup>1,2</sup>, T. van Wezel<sup>1</sup>, B. E. W. M. van den Akker<sup>1</sup>, M. Ventayol Garcia<sup>1</sup>, D. Ruano<sup>1</sup>, C. M. J. Tops<sup>3</sup>, A. Wagner<sup>4</sup>, T. G. W. Letteboer<sup>5</sup>, E. B. Gómez-García<sup>6</sup>, P. Devilee<sup>7</sup>, J. T. Wijnen<sup>2,3</sup>, F. J. Hes<sup>3</sup>, H. Morreau<sup>1</sup>;

<sup>1</sup>Department of Pathology, Leiden University Medical Center, Leiden, Netherlands,

<sup>2</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands,

<sup>3</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands,

<sup>4</sup>Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands,

<sup>5</sup>Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands,

<sup>6</sup>Department of Clinical Genetics, University Hospital Maastricht, Maastricht, Netherlands,

<sup>7</sup>Department of Pathology, Leiden University Medical Center

Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

Introduction: Many suspected Lynch Syndrome (sLS) patients who lack mismatch repair (MMR) germline gene variants and MLH1 or MSH2 hypermethylation are currently explained by somatic MMR gene variants or, occasionally, by germline POLE variants.

Materials and Methods: To further investigate unexplained sLS patients, we analyzed leukocyte- and tumor DNA of a cohort of 62 sLS patients using gene panel sequencing including the POLE, POLD1 and MMR genes.

Results: Forty tumors showed either one, two or more somatic MMR variants predicted to affect function. Nine sLS-tumors showed an ultramutated phenotype and were found to carry germline- (n=2) or somatic variants (n=7) in the POLE/POLD1 exonuclease domain (EDM). Six of these POLE/POLD1-EDM mutated tumors also carried somatic MMR variants. Four of these tumors showed low or high microsatellite instability.

Conclusions: Our findings suggest that faulty proofreading may result in

loss of MMR and thereby in microsatellite instability. While literature mainly addresses POLE/POLD1 variant in microsatellite stable tumors, somatic POLE/POLD1 variants in SLS patients are likely to be overlooked. Our results further emphasize the importance of POLE/POLD1 germline and somatic screening in unexplained MSI-H and MMR-deficient tumors. This work was supported by the Dutch Cancer Society under study number UL2012-5542.

#### P12.182

#### Promoter methylation profiling of head and neck squamous cell carcinoma

S. Stangler Herodez<sup>1</sup>, B. Zagradišnik<sup>1</sup>, B. Cizmarevic<sup>2</sup>, N. Kokalj Vokac<sup>1,3</sup>,

<sup>1</sup>Laboratory of Medical Genetics, UKC Maribor, Maribor, Slovenia, <sup>2</sup>Department of Otorhinolaryngology and Maxillofacial Surgery, UKC Maribor, Maribor, Slovenia,

<sup>3</sup>University of Maribor, Faculty of Medicine, Maribor, Slovenia.

Introduction Silencing of tumor suppressor genes by DNA promoter hypermethylation is an early event in carcinogenesis and a potential target for personalized cancer treatment. In head and neck squamous cell carcinoma (HNSCC) limited knowledge is available about the role of promoter hypermethylation in cancer progression.

Materials and methods In present work we have investigated DNA methylation of 24 different tumor suppressor genes in 62 patients with various stages of HNSCC (34 oral squamous cell carcinoma (OSCC) and 28 oropharyngeal squamous cell carcinoma (OPSCC)) with methylation specific multiplex ligation-dependent probe amplification (MS-MLPA). We analyzed genomic DNA extracted from homogenized fresh-frozen HNSCC tissue specimens. Results Promoter hypermethylation ( $\geq 2$  genes) was observed in 48 of the 62 cases (77%). HNSCC showed frequent promoter hypermethylation in ATM (67%), TIMP3 (67%), RARB (65%), DAPK1 (55%), CDH13 (55%), APC (36%) and IGSF4 (36%). Total number of hypermethylated genes in OSCCs did not differ statistically from the number of total hypermethylated genes in OPSCCs ( $P=0.7866$ ). Promoter hypermethylation in OSCC didn't reveal statistically significant differences between the early and advanced tumor stage ( $P=0.5081$ ). Similarly OPSCC early onset cases didn't differ from advanced stage cancers ( $P=0.6911$ ).

Conclusion Promoter methylation profiling of HNSCC using MS-MLPA identified ATM, TIMP3, RARB, DAPK1, CDH13, APC and IGSF4 as frequent epigenetic events. Observed findings permit the identification of the nature of the tumor but they don't allow the differentiation according the tumor stage. Validation of these findings in larger HNSCC units would support these genes as relevant biomarkers for cancer management.

#### P12.183

#### Functional analysis of RAD51B and RAD23B SNPs using Circular Chromosome Conformation Capture (4C) in human prostate cell lines.

C. Cieza-Borrell<sup>1</sup>, S. Stephens<sup>1</sup>, Q. Karlsson<sup>1</sup>, R. Eeles<sup>1,2</sup>, Z. Kote-Jarai<sup>1</sup>;

<sup>1</sup>The Institute of Cancer Research, Sutton, United Kingdom, <sup>2</sup>The Royal Marsden Hospital NHS Trust, London, United Kingdom.

INTRODUCTION: Prostate Cancer (PrCa) is the most frequently diagnosed cancer among men in developed countries. Genome-wide association studies (GWAS) have identified over 100 common, low penetrance PrCa susceptibility variants, some near or within RAD51B and RAD23B genes. Our aim is to better understand associations between these variants and PrCa risk, analysing their interactions with the whole genome, by Circular Chromosome Conformation Capture (4C).

MATERIAL AND METHODS: Genomic interactions of 10 single nucleotide polymorphisms (SNPs), selected from previous GWAS and fine mapping studies, were investigated in five human prostate epithelial cell lines (LNCaP, DuCaP, PC3, PNT1a and RWPE-1) presenting diverse phenotypes and genotypes. 4C assay design was carried out using 4C Primer Designer for 4C viewpoints program. Libraries were run on an Illumina HiSeq 2500 (RAPID chemistry). Bioinformatics analysis was performed using the program 4C-ker.

RESULTS: Preliminary results show numerous interactions between analysed SNPs and the rest of the genome. Of particular interest, a significant cis association was observed, in all three adenocarcinoma cell lines, between rs7141529 and rs767127 (both RAD51B SNPs) with FUT8. Interaction between rs767127 and the RAD51B promoter region was also detected. In addition rs767127 also presented cis interactions with FOS, YY1 and EVL. We will also present data on the RAD23B SNPs as analysis is currently ongoing. CONCLUSION: 4C analysis showed interactions in adenocarcinoma prostate cell lines between rs7141529 and rs767127 and various regions of high interest due to their implications in cancer development. Further analysis, using Chromosome Conformation Capture (3C), will be conducted to validate results.

#### P12.185

#### HOXB13 gene exon 1-2 mutations among patients with prostate cancer

D. Alptekin<sup>1</sup>, M. A. Erkoç<sup>1</sup>, F. Gökçalp<sup>2</sup>, M. İzmirli<sup>3</sup>, Y. Bayazıt<sup>2</sup>, N. S. İlgaç<sup>1</sup>, N. Satar<sup>2</sup>, H. Ü. Lüleyap<sup>1</sup>;

<sup>1</sup>Cukurova University, Medical Faculty, Dept. of Medical Biology, Adana, Turkey,

<sup>2</sup>Cukurova University, Medical Faculty, Dept. of Urology, Adana, Turkey, <sup>3</sup>Mustafa Kemal University, Medical Faculty, Dept. of Medical Biology, Hatay, Turkey.

Introduction: Prostate cancer susceptibility gene is located on chromosome 17q21-22 which is reported to be associated with Homeobox (HOX) gene family. HOX genes' proteins resulting from transcription of this gene is associated with prostate cancer which plays important role in regulation of Androgen receptors. One of the most important members of HOX family is HOXB13. G84E codon mutation of HOXB13 is reported to be associated with prostate cancer. This study aimed to investigate relationship between prostate cancer and HOXB13 exon 1-2 mutations.

Materials and Methods: In this study, 64 prostate cancer patients are involved. After DNA isolation, sequence analysis is performed with Big Dye Cycle sequencing PCR technique. HOXB13 exon 1-2 are amplified and sequence analysis is performed with ABI PRISM® 3100 Genetic Analyzer.

Results: We couldn't determine any G84E mutation. However, 44 normal homozygote(CC), 16 heterozygote(CT) and 4 mutant homozygote(TT) genotypes have been found in 366 base position of the coding region of HOXB13 gene. In addition, 63 normal homozygote and 1 mutant heterozygote(CT) genotypes have been found in 411 position of coding region. Also, 45 normal homozygote(TT), 12 heterozygote(TC) and 7 mutant(CC) genotypes have been found. There was no mutation in exon 2.

Conclusions: Previously described 366 and 513 base mutations have been thought as a polymorphism because of no amino acid substitution in protein of HOXB13. Due to this reason, there is no association between these mutations and prostate cancer. However, it has been thought that there is an association between 411 base mutations and prostate cancer.

#### P12.186

#### The prognostic importance of the MGMT and RAR $\beta$ gene hypermethylation in Primary Glioblastoma

R. Kalkan<sup>1</sup>, E. I. Atlı<sup>2</sup>, M. Özdemir<sup>3</sup>, H. E. Aydin<sup>3</sup>, A. Arslantaş<sup>3</sup>, S. Artan<sup>3</sup>;

<sup>1</sup>Near East University, Nicosia, Cyprus, <sup>2</sup>Trakya University, Turkey, Turkey, <sup>3</sup>Eskişehir Osmangazi University, Eskişehir, Turkey.

Background: We screened RAR $\beta$  and MGMT methylation in 40 primary glioblastoma multiforme (GBM) sample. We used MS-HRM for evaluation of methylation levels in primary GBM samples. This study indicates a potential prognostic value for GBM treatment planning.

Results: MGMT methylation was detected in 13 of the 40 patients (32,5%) and the overall survival time is 19 months for methylated MGMT patients, it is 15 months for unmethylated MGMT patients. MGMT methylation did not significantly associate ( $p > 0,05$ ) with overall survival time MGMT-promoter methylation did not correlate with overall survival (OS;  $p > 0,05$ ).

RARB methylation was detected in 24 of the 40 patients (60%). The overall survival time of the patients with methylated RAR $\beta$  was 19 months, and nonmethylated RARB was 15 months. The statistical analysis shows that the patients who received both chemotherapy and radiotherapy treatment combined had a survival time of 25 months. The patients who received only radiotherapy or had no treatment protocol had a survival time between 15-20 months, which demonstrates a significant difference ( $P < 0,05$ ). Statistical difference was observed between the survival times of the patients receiving both radiotherapy and chemotherapy treatment and the cases with no applied treatment protocol.

Conclusion: In summary, our results demonstrated that the positive correlation between RAR $\beta$  methylation status and radio/chemotherapy treatment. Furthermore MGMT promoter methylation had no prognostic value and lower frequency in primary glioblastomas.

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#### P12.188

#### Comprehensive Analyses of the Variants of the Oncogene, FAT10 in Hepatocellular Carcinoma (HCC) patients

C. G. Lee<sup>1,2,3</sup>, C. C. T. Sim<sup>2</sup>, J. Yu<sup>1,2</sup>, S. S. Theng<sup>1,2</sup>, S. S. Chong<sup>2</sup>;

<sup>1</sup>National Cancer Centre, Singapore, Singapore, <sup>2</sup>National University of Singapore, Singapore, Singapore, <sup>3</sup>Duke-NUS, Singapore, Singapore.

Hepatocellular Carcinoma (HCC) is a deadly disease due to its late presentation and limited therapeutic options. Our laboratory previously reported FAT10 as an oncogene that is highly up-regulated in HCC.

FAT10 gene is 4,314 bp long located on chromosome 6. Since FAT10 is an

oncogene, and mutations/polymorphisms can influence susceptibility to cancer; we hypothesized that mutations/polymorphisms within the FAT10 gene will modulate the expression / function of the FAT10 protein and be associated with differential risk for the development of HCC and/or differences in clinical outcome.

The architecture of polymorphisms in the FAT10 gene is noteworthy. For such a small gene like FAT10 with an open reading frame of ~500 bp, it has ~9 single-nucleotide polymorphisms within its coding region of which 8 were non-synonymous and 4 were also non-conservative changes. This observation suggests that many of these polymorphisms can potentially affect function and perhaps even modulate its tumorigenic properties. Interestingly, the haplotype of polymorphisms in the different ethnic population as well as between healthy individuals and HCC patients are also different suggesting that variability of the polymorphisms in the different ethnic groups may account for variability in susceptibility to cancer development. The polymorphisms within the coding region had been recapitulated in cells and characterized.

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#### P12.189

#### Functional characterization of DNA variants from exons 17 and 18 of the BRCA2 gene

*E. Fraile-Bethencourt<sup>1</sup>, B. Díez-Gómez<sup>1</sup>, V. Velásquez-Zapata<sup>2</sup>, A. Acedo<sup>3</sup>, D. Sanz<sup>4</sup>, C. Hernández-Moro<sup>5</sup>, G. Marcos<sup>6</sup>, M. Infante<sup>1</sup>, M. Durán<sup>1</sup>, E. Velasco<sup>1</sup>;*

<sup>1</sup>Instituto de Biología y Genética Molecular (CSIC/UVa), Valladolid, Spain, <sup>2</sup>University of Antioquia, Medellín, Colombia, <sup>3</sup>AC-GEN Reading Life SL, Valladolid, Spain, <sup>4</sup>University College Cork, Cork, Ireland, <sup>5</sup>EuroTarget, Centro integral de oncología Clara Campal, Madrid, Spain, <sup>6</sup>Hospital Universitario Río Hortega, Valladolid, Spain.

A large fraction of pathogenic BRCA2 variants impairs mRNA splicing in hereditary breast/ovarian cancer. Missense, synonymous or in frame-deletion/insertion variants are usually classified as variants of uncertain significance. The best method to identify splicing aberrations is based on the study of patient RNA, but that is often difficult to obtain. Minigene-based technology is an alternative approach to test candidate splicing variants without the need of patient samples. To study this process we constructed a large minigene in the pSAD plasmid with exons 14 to 20 (MGBR2\_ex14-20) to keep the genomic context. Here, we focus on exons 17 y 18 because of the atypical GC donor in exon 17. The intronic GT dinucleotide is the most conserved element of the donor splice signal. However, in a small fraction of the donor sites (<1%), GT is replaced by GC that are rather located in alternatively spliced introns. A total of 226 variants were analysed with NN-Splice and HSF so that 48 of them were selected to assay. They were introduced into MGBR2\_ex14-20 by site-directed mutagenesis. Minigenes were transfected into HeLa and MCF7 cells to examine their transcripts. We found that 22 variants (45.8%) produced abnormal transcripts. Moreover, micro-deletion assays showed that the 3' region of exon 17 and the 5' of exon 18 were essential for exon recognition and might contain splicing enhancer sequences. Actually, five reported variants mapping in these intervals disrupted splicing.

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#### P12.190

#### Relative gene amplification analysis of FGFR1, MET, DCUN1D1 and BCL9 gene in squamous cell carcinomas of the lung

*A. A. Psaraki, I. Chatziandreou, I. Mourkioti, P. Korkolopoulou, E. Patsouris, A. A. Saetta; National and Kapodistrian University of Athens, Medical school, Department of Pathology, Athens, Greece.*

**Background:** The discovery of new biomarkers and therapeutic targets is of great importance for the clinical benefit of NSCLC patients. Since gene amplification is regarded as an important oncogenic target in lung Squamous Cell Carcinoma, we performed relative amplification analysis of FGFR1, MET, DCUN1D1 and BCL9 genes in lung squamous cell carcinomas, and searched for correlations with KRAS mutations and clinicopathological characteristics.

**Materials and Methods:** DNA was isolated from 73 lung Squamous Cell Carcinoma FFPE tissues. Real time- PCR using GAPDH as a reference gene was performed and Relative gene amplification was calculated using the normalized ratio. Statistical analysis was performed using SPSS v21 statistical package.

**Results:** Relative gene amplification was detected as follows: FGFR1 12.3%, MET 15.1%, DCUN1D1 38.4% and BCL9 43.8%. KRAS was found mutated in 2 cases, whereas no EGFR mutations or ALK rearrangements were detected. In addition, DCUN1D1 and BCL9 were correlated with low tumor differentiation. A subset of cases 22% displayed co-amplification of two genes.

**Discussion:** The percentages of gene amplification of suggested new druggable target genes FGFR1, MET, DCUN1D1 and BCL9 are in accordance with recent findings. DCUN1D1 and BCL9 relative amplification emerged as possible markers of low tumor differentiation. Interestingly, a high percentage of the samples showed co-amplification of at least 2 genes.

#### P12.191

#### High complexity libraries and targeted NGS panels to access sequencing information of limiting samples

*M. Hymes, C. Schumacher, S. Sandhu, L. Kurihara, S. Chupretta, J. Irish, J. Laliberte, J. Lenhart, J. RoseFigura, T. Harkins, V. Makarov; Swift Biosciences, Ann Arbor, MI, United States.*

Targeted sequencing by hybridization capture provides a cost effective method for performing Next Generation Sequencing (NGS) on selected regions of the genome. While formalin-fixed, paraffin-embedded (FFPE) samples are utilized in targeted sequencing, the amount and quality of their DNA is typically compromised. Library preparations that maximize complexity and coverage uniformity across the genome are especially valuable for these limiting samples, which would otherwise produce high duplicate rates and poor coverage metrics when pursuing the deep sequencing required to call somatic variations. Here we present a library preparation method to enable high quality libraries for target enrichment, utilizing a variety of sample types.

For hybridization capture, libraries were constructed from 1-100 ng of Coriell HapMap samples, clinical FFPE, circulating cell-free DNA (cfDNA) and Horizon Discovery reference DNA using a novel library preparation method. Hybridization capture was performed using commercially available panels from three different vendors.

A Pan-Cancer Panel yielded <3% duplicates and ≥40X coverage on 10 ng and 100 ng samples of Coriell DNA, while maintaining 30X coverage at 1 ng inputs. Additionally, kidney samples that underwent formalin fixation for 6, 24, and 48 hours were compared to fresh frozen. While fresh frozen samples performed equivalently to Coriell DNA samples, formalin fixed samples demonstrated <8% duplicates at 10 ng and 100 ng, and <45% duplicates and ≥22X coverage at 1 ng, illustrating the effects of fixation on DNA quality. Somatic variant calling down to 1% allele frequency was evaluated at various DNA quantities with reference samples.

#### P12.192

#### Next generation sequencing of patients with familial papillary thyroid carcinoma and their relatives

*D. N. Nikolova<sup>1</sup>, Z. Hammoudeh<sup>1</sup>, L. Balabanski<sup>2</sup>, S. Ivanov<sup>2</sup>, R. Vazharova<sup>2</sup>, S. Weidner<sup>3</sup>, D. Toncheva<sup>1,2</sup>,*

<sup>1</sup>Medical University, Sofia, Bulgaria, <sup>2</sup>Genomic Laboratory, SBALGAR "Malinov", Sofia, Bulgaria, <sup>3</sup>Bern University Hospital, Bern, Switzerland.

**Introduction:** The incidence of thyroid cancer is steadily growing worldwide. It develops and progresses through accumulation of genetic alterations the most common being point mutations in BRAF and RAS genes and RET/PTC rearrangements, all of which activate the MAPK pathway. We used NGS technology for analysis of potentially pathologic mutations predisposing to the development of familial papillary thyroid carcinoma (FPTC) in affected families.

**Materials and Methods:** DNA from peripheral blood was isolated from 23 individuals (11 patients with FPTC, 2 patients with other thyroid disorders and 10 unaffected relatives). We used TruSight Cancer sequencing panel (Illumina) targeting 94 genes and 284 SNPs.

**Results:** In our study we selected 44 missense variants, four of which are pathogenic or potentially pathogenic. In our first family we determined the variant FANCD2\_c.1137G>T associated with Fanconi anemia. In the second family we proved three variants associated with Fanconi anemia: FANCA\_p.Ser1088Phe, FANCD2\_c.1137G>T and SLX4\_p.Pro245Leu. In the third family we defined the same Fanconi anemia variants FANCA\_p.Ser1088Phe, FANCD2\_c.1137G>T and the variant HOXB13\_p.Gly84Glu predisposing to prostate and colorectal cancer. In the fourth family again the same HOXB13\_p.Gly84Glu was carried.

**Conclusion:** Mutations in three of the genes (FANCA, FANCD2, and SLX4) belong to Fanconi anemia complementation group and cause cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. Mutations in the fourth gene

HOXB13 predispose to carcinomas. The results suggest a possible role of these mutations in causing genetic predisposition to familial papillary thyroid carcinoma.

## P12.193

### Mutations in MYO1F cause familial non-medullary thyroid cancer (FNMTC)

*C. Diquigiovanni<sup>1</sup>, C. Bergamini<sup>2</sup>, C. Evangelisti<sup>3</sup>, A. Costanzini<sup>2</sup>, F. Isidori<sup>1</sup>, H. Ambunathan<sup>4</sup>, A. Bowcock<sup>4</sup>, R. Casadio<sup>5</sup>, G. Babbi<sup>5</sup>, K. J. Rhoden<sup>1</sup>, G. Lenaz<sup>2</sup>, R. Fato<sup>2</sup>, M. Seri<sup>1</sup>, G. Romeo<sup>1</sup>, E. Bonora<sup>1</sup>;*

<sup>1</sup>Department of Medical and Surgical Sciences, St. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy, <sup>2</sup>Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, <sup>3</sup>Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, <sup>4</sup>National Heart and Lung Institute, Imperial College, London, United Kingdom, <sup>5</sup>Biocomputing Group, University of Bologna, Bologna, Italy.

Familial Non-Medullary Thyroid Cancer (FNMTC) accounts for 5-7% of all NMTC. Since these patients develop more aggressive tumours with worse outcomes compared to the sporadic counterparts, the identification of the susceptibility genes is crucial for treatment and surveillance.

Whole exome sequencing in a multigenerational family affected by FNMTC with oncocytic features, revealed a novel heterozygous mutation in MYO1F gene, encoding for an unconventional myosin and mapping to chromosome 19p13.2, where our group previously identified a predisposing locus. Cell lines stably expressing mutant MYO1F showed more mitochondria, an altered mitochondrial network and increased levels of endogenous and extracellular ROS, compared to cells overexpressing the wild-type protein or the empty vector. The mutation also conferred a significant increase in colony formation, invasion and anchorage-independent growth, along with an over-activation of ERK1/2 pathway. These results suggest that the MYO1F variant is the mutation underlying the linkage locus previously mapped at chromosome 19p13.2.

192 independent FNMTC patients were screened to identify additional predisposing variants in MYO1F. We identified a rare silent change (rs184748543G>A) altering an Exonic Sequence Enhancer (ESE) in one patient and his affected sister. In vitro transcription analysis using a mini-gene containing-plasmid showed that the rare A allele determines an exon skipping, leading to an in-frame deletion of 43 amino acids, which alters the ATP-binding domain in MYO1F.

These results suggest that rare variants in MYO1F may predispose to FNMTC susceptibility.

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## P12.194

### Novel germline TP53 mutations in the Swedish constitutional TP53 cohort

*P. Kharaziha<sup>1,2</sup>, C. Larsson<sup>1</sup>, E. Tham<sup>3,4</sup>, S. Bajalica-Lagercrantz<sup>1,2</sup>;*

<sup>1</sup>Department of Oncology-Pathology, Cancer Center Karolinska (CCK), Karolinska Institutet, Stockholm, Sweden, <sup>2</sup>Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, <sup>3</sup>Clinical Oncology, Karolinska University Hospital, Stockholm, Sweden, <sup>4</sup>Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

**Introduction:** Individuals with a constitutional TP53 mutation have a high risk of cancer in childhood and/or early adulthood, but show a large phenotypic variation. In some families, pediatric tumors are more frequent while in others only breast cancer is seen. The underlying cause for this variation is unknown. However, this question is of utmost importance for which surveillance should be offered to mutation carriers. It is therefore a large need for further molecular characterization of the genomic landscape with regard to both the TP53-mutations, as well as of genetic modulating factors, in order to understand the genotype-phenotype correlation.

**Materials and Methods:** We have generated cDNA expression constructs for all nine novel germline TP53 mutants.

**Results:** In the Swedish cohort of 32 families, we found 25 different mutations in the TP53 gene. Nine have not been reported previously as germline mutations according to the HGMD or IARC databases. Five of these have not even been reported as somatic mutations in the IARC or cosmic databases and are thus truly novel. Transfection in p53 null H1299 cells have demonstrated a wide range of activities among the constructs in terms of induction of apoptosis in response to 12 G irradiation, as well as variable impact on the cell cycle regulation.

**Conclusions:** We showed that all novel mutations were express stably in cell lines and decrease spontaneous apoptosis and have a poorer response to therapy which indicate that affected individual has higher risk of tumorige-

nosis and less response to radiotherapy.  
Grant: BRECT

## P12.195

### Mutational biases drive elevated rates of substitution at regulatory sites across cancer types

*V. B. Kaiser, M. S. Taylor, C. A. Semple;*

*IGMM, MRC Human Genetics Unit, University of Edinburgh, Edinburgh, United Kingdom.*

Disruption of gene regulation is known to play major roles in carcinogenesis and tumour progression. Here, we comprehensively characterize the mutational profiles of diverse transcription factor binding sites (TFBSs) across 1,574 completely sequenced cancer genomes encompassing 11 tumour types. We assess the relative rates and impact of the mutational burden at the binding sites of 81 transcription factors (TFs), by comparing the abundance and patterns of single base substitutions within putatively functional binding sites to control sites with matched sequence composition. There is a strong (1.43-fold) and significant excess of mutations at functional binding sites across TFs, and the mutations that accumulate in cancers are typically more disruptive than variants tolerated in extant human populations at the same sites. CTCF binding sites suffer an exceptionally high mutational load in cancer (3.31-fold excess) relative to control sites, and we demonstrate for the first time that this effect is seen in essentially all cancer types with sufficient data. The sub-set of CTCF sites involved in higher order chromatin structures has the highest mutational burden, suggesting a widespread breakdown of chromatin organization. However, we find no evidence for selection driving these distinctive patterns of mutation. The mutational load at CTCF-binding sites is substantially determined by replication timing and the mutational signature of the tumor in question, suggesting that selectively neutral processes underlie the unusual mutation patterns. Pervasive hyper-mutation within transcription factor binding sites rewrites the regulatory landscape of the cancer genome, but it is dominated by mutational processes rather than selection.

## P12.196

### Contribution of misreading Serine tRNAs to tumor growth in vivo

*M. R. Santos<sup>1,2</sup>, P. M. Pereira<sup>1,2,3</sup>, S. Varanda<sup>1,2,3</sup>, J. Carvalho<sup>2,3</sup>, M. Azevedo<sup>2</sup>, D. D. Mateus<sup>2</sup>, N. Mendes<sup>2,3</sup>, P. Oliveira<sup>2,3</sup>, M. T. Pinto<sup>2,3</sup>, R. Bordeira-Carrizo<sup>2,3</sup>, F. Carneiro<sup>2,3,4</sup>, M. A. S. Santos<sup>1</sup>, C. Oliveira<sup>2,3,4</sup>;*

<sup>1</sup>Department of Medical Sciences and Institute of Biomedicine – iBiMED, University of Aveiro, Aveiro, Portugal, <sup>2</sup>Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal, <sup>3</sup>Instituto de Investigação e Inovação em Saúde, Porto, Portugal, <sup>4</sup>Dept. Pathology and Oncology, Faculty of Medicine, University of Porto, Porto, Portugal.

**Introduction:** Upregulation of protein synthesis, deregulation of tRNA expression and amino acid starvation are common features of cancer. The occurrence of these events raises the hypothesis that translational fidelity is compromised in tumors (1,2). However, the relevance of tRNA misreading in cancer is still unknown. To clarify the role of tRNA misreading in cancer development, we expressed misreading tRNAs in a near-normal cell line and studied UPR and cancer-associated signaling pathways.

**Materials and Methods:** NIH3T3 cell line was stably transfected with pIRE2-DsRED plasmids containing the tRNASer(WT), tRNAs that misincorporate Serine(Ser) at Alanine(Ala)-GCU or Leucine(Leu)-CUU sites and also an empty plasmid. Cell lines were injected in mice and their tumorigenic potential was evaluated. tRNA expression both in cell lines and in tumors was determined by SNaPshot sequencing. Alterations in UPR and cancer-related pathways were accessed by western blot.

**Results and Conclusions:** We report an unexpected role for misreading tRNAs in tumor growth. Our data show that expression of misreading tRNAs produce tumors with similar growth rate to K-ras-induced tumors. Remarkably, expression of misreading tRNAs increases in vivo, suggesting advantageous features of this phenotype. Our results also showed that Akt and UPR pathways are activated in a microenvironment-dependent manner. Accumulating evidence has demonstrated that UPR activation and decrease in translation fidelity are required for cancer cells to maintain malignancy and acquire therapy resistance, suggesting that compromising translation fidelity may select adaptative mutations. Our results support the hypothesis that mistranslation phenotypes have a role in tumor growth, worth to explore further.

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## P13 Basic mechanisms in molecular and cytogenetics

### P13.01

#### Interstitial 10q21.1-q23.31 duplication due to a meiotic recombination of a paternal balanced complex rearrangement: cytogenetic and molecular fine characterization

V. Orlando<sup>1,2</sup>, V. Alesi<sup>1</sup>, C. Surace<sup>1</sup>, D. Pompili<sup>1</sup>, F. Restaldi<sup>1,2</sup>, M. Dentici<sup>3</sup>, M. Digilio<sup>3</sup>, A. Novelli<sup>1</sup>;

<sup>1</sup>Laboratory of Medical Genetics, „Bambino Gesù“ Children’s Hospital, Roma, Italy,

<sup>2</sup>Department of Experimental Medicine, Sapienza University, Rome, Italy, <sup>3</sup>Medical Genetics, „Bambino Gesù“ Children’s Hospital, Roma, Italy.

Chromosome structural rearrangements, such as translocations, inversions and insertions, are aberrations involving one or more chromosomes and are relatively common in human population. They are defined as “balanced” if they do not lead to a gain or a loss of genetic material and “unbalanced” when they are associated with duplications or deletions at breakpoints or as a consequence of malsegregation during cell division. Rearrangements associated with more than two breakpoints are rare and they have a higher probability of a pathological outcome, because of gene disruption or of a not correct segregation pattern.

We describe a complex rearrangement involving chromosomes 7 and 10 with 4 different breakpoints in an asymptomatic man and in his son, the latter presenting with developmental delay, speech delay, growth retardation, microcephaly, hypospadias and dysmorphic features. The child inherited both the paternal derivatives but the rearranged chromosome 10 also harboured an interstitial duplication due to a recombination event. During paternal meiosis, two different crossing-over occurred upstream and downstream the 10q21.1q23.31 region, between the derivative 10 chromosome and the normal chromosome 10 homologous, bringing back a copy of the missing portion in its original position. As a consequence the child harboured a 32.4 Mb duplication of chromosome 10, characterized by array-CGH. The involved region completely overlaps the critical 10q22.3q23.3 region, whose duplication is described in association with microcephaly, developmental delay, speech delay, growth retardation and dysmorphic features.

### P13.02

#### The malsegregation of a cryptic paternal chromosomal rearrangement results in two 5q noncontiguous microduplications: evidences of a reciprocal phenotype of the 5q31.3 deletion syndrome.

S. Loddò<sup>1</sup>, V. Alesi<sup>1</sup>, M. G. Giuffrida<sup>2</sup>, M. T. Liambo<sup>1</sup>, M. Grispoli<sup>1</sup>, L. Bernardini<sup>2</sup>, M. C. Digilio<sup>3</sup>, A. Novelli<sup>1</sup>;

<sup>1</sup>Laboratory of Medical Genetics, „Bambino Gesù“ Children’s Hospital, Rome, Italy,

<sup>2</sup>Mendel Laboratory, Casa Sollievo della Sofferenza Hospital, IRCCS, Rome and San Giovanni Rotondo, Italy, <sup>3</sup>Medical Genetics, „Bambino Gesù“ Children’s Hospital, Rome, Italy.

The great majority of apparently balanced translocation are associated with normal phenotype, although a small percentage of carriers shows abnormal phenotypes. One of the mechanisms proposed to explain this phenomenon is the presence of a cryptic complex chromosomal rearrangement (CCR). CCRs are structural anomalies involving at least three chromosomes or three breakpoints. The use of FISH assays and DNA microarray technologies for the characterization of CCRs has allowed the identification of cryptic imbalances at breakpoints sites.

We report a girl, carrier of an apparently balanced translocation t(3;11) inherited from her healthy father, showing psychomotor delay, dysmorphic features, microcephaly, hypertrichosis, hypotonia, seizures and precocious puberty. Array-CGH analysis highlighted the duplication of two non contiguous regions of the long arm of a chromosome 5, extended 6 Mb (5q31.1q31.3) and 3,2 Mb (5q34) respectively. FISH assay, also performed on paternal chromosomes, revealed a more complex rearrangement involving chromosomes 3, 5 and 11. The child inherited the derivative chromosomes 3 and 11 and the normal chromosome 5, therefore showing the two duplicated regions of 5q as consequence of malsegregation of the paternal balanced CCR.

Of note, 5q31.1q31.3 duplication overlapped the critical region of 5q31.3 microdeletion syndrome characterized by hypotonia, seizures, developmental delay and facial dysmorphisms. Our patient shared many of these features, suggesting the existence of a distinct phenotype associated with the reciprocal duplication of the 5q31.3 microdeletion syndrome.

### P13.03

#### Genetic studies reveal five new mutations in the CP gene in patients affected by autosomal recessive ceruloplasminemia

A. Barqué<sup>1</sup>, A. Negro<sup>1</sup>, N. Wood<sup>2</sup>, E. Jové-Buxeda<sup>3</sup>, J. Sanchez-Delgado<sup>4</sup>, B. Burnyte<sup>5</sup>, N. Baena-Díez<sup>6</sup>, J. Limón<sup>7</sup>, F. Fuster<sup>1</sup>, J. Aranda<sup>1</sup>, M. Sanchez<sup>1</sup>;

<sup>1</sup>Diagnostics in Iron Metabolism Diseases (D-IRON), Josep Carreras Leukaemia Research Institute (JCI), Badalona, Spain, <sup>2</sup>National Hospital for Neurology and Neurosurgery, London, United Kingdom, <sup>3</sup>Internal Medicine Department, University Hospital Parc Taulí, Sabadell, Spain, <sup>4</sup>Hepatology and Digestive Department, University Hospital Parc Taulí, Sabadell, Spain, <sup>5</sup>Center for Medical Genetics, Hospital Santarískiu Klinikos, Vilnius, Lithuania, <sup>6</sup>Genetic Department, University Hospital Parc Taulí, Sabadell, Spain, <sup>7</sup>Department of Biology and Genetics, Medical University of Gdańsk, Gdańsk, Poland.

Ceruloplasminemia is a rare autosomal recessive genetic disease with an adult onset characterized by iron-refractory anemia, retinal degeneration, diabetes mellitus and various neurological symptoms (including ataxia, involuntary movements, parkinsonism, depression and cognitive dysfunction) due to iron accumulation in the brain and viscera. It is caused by the absence of ceruloplasmin ferroxidase activity due to mutations in the ceruloplasmin (CP) gene.

Diagnosis is based on the absence of serum ceruloplasmin and some combination of low serum copper concentration, low serum iron concentration, high serum ferritin concentration as well as hepatic iron overload. The diagnosis is strongly supported by characteristic MRI findings of abnormal low intensities reflecting iron accumulation on the brain and liver. Genetic testing can confirm the diagnosis.

Treatment is based on intravenous and oral iron chelators, which have been associated with improvement in diabetes and neurological symptoms. Combined intravenous desferrioxamine and fresh-frozen human plasma (FFP) is effective in decreasing iron content in the liver. Antioxidants such as vitamin E and oral administration of zinc may prevent tissue damage.

Here we will discuss the clinical and genetic aspects of 6 new cases of ceruloplasminemia, (including two young case <40 years old) presenting 5 previously not described mutations (one missense, three frameshifts and one intronic mutation).

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### P13.04

#### Evidence of high pathogenicity of a de novo mutation in polycystin-1 gene

J. F. Rocha, R. Pinto Leite, M. Martins, M. Fructuoso, T. Morgado;

Centro Hospitalar de Tras-os-Montes e Alto Douro, Vila Real, Portugal.

**Introduction:** Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the main inherited cause of Chronic Kidney Disease (CKD). Mutations in polycystin-1 gene are responsible for 85% of the cases. The variant c.7837\_7839del (p.Leu2613del) is an in-frame deletion of three nucleotides, resulting in the deletion of one amino acid in polycystin-1. It was already described in Human Gene Mutation Database but its pathogenicity was so far undetermined.

**Cases Presentation:** we describe an ADPKD family with several members affected by a de novo genetic mutation. CKD was diagnosed in the mother at 38y, who started hemodialysis at 41y. The first son presents CKD at 33y. The second son had CKD since 24y, having started hemodialysis at 28y. A niece had the diagnosis at 13y without Renal Failure yet. The variant c.7837\_7839del (p.Leu2613del) was found in all patients. The mutation was not found in a daughter who did not present disease criteria. Awaiting genetic study are sixteen relatives with ADPKD (6 started dialysis early).

**Conclusion:** The mutation variant c.7837\_7839del in the polycystin-1 gene was the only one found in four affected family members (three in first degree) and was absent in a relative without the disease, confirming its pathogenicity. Many cases developed early severe CKD, in favor of a mutation with high pathogenicity. The early and severe manifestations of ADPKD make relatives seek reliable prenatal diagnosis. Therefore, every new case of a rare genetic mutation should be reported in order to obtain a more precise genotype/phenotype correlation, improving risk evaluation and genetic counseling.

## P13.05

### Association of FAS promoter gene polymorphism in alopecia areata

E. Ensari<sup>1</sup>, O. Ates<sup>1</sup>, G. Kalkan<sup>2</sup>;

<sup>1</sup>Gaziosmanpasa University, Medical Faculty, Department of Medical Biology, Tokat, Turkey, <sup>2</sup>Yildirim Beyazit University, Medical Faculty, Department of Dermatology, Ankara, Turkey.

**Introduction:** Alopecia areata (AA) is a T cell-mediated autoimmune disease characterized non-scarring, patchy loss of hair in the scalp and elsewhere. Onset of AA may occur at any age. Incidence is equally distributed across races and genders. Its etiopathogenesis is exactly uncertain yet. AA has a multifactorial etiology that both several genes and environmental factors come together with a different weight in triggering the pathology. Fas cell surface death receptor (FAS), a member of the tumor necrosis factor (TNF) receptor super-family, is a transmembrane receptor involved in apoptotic signal transmission in many cell types and play essential roles in many human autoimmune diseases. The aim of this study was to evaluate the possible association between the 1377 G>A promoter polymorphism in the FAS gene and risk of AA.

**Materials and methods:** The study group included 200 patients with AA and 165 healthy volunteers. Genotyping for the FAS-1377 G>A polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RLFP) method.

**Results:** A statistically significant difference was observed between patients and controls according to genotype and alleles frequencies of FAS gene 1377 G>A polymorphism ( $p = 0.007$  and  $p = 0.000$ , OR 1.98, 95 % CI 1.35-2.92, respectively).

**Conclusion:** According to our findings, the FAS-1377 G>A polymorphism is associated with AA. The presence of the A allele is thought to protective to AA. This work was supported by Gaziosmanpasa University, Unit Of Scientific Research Projects

## P13.06

### Identification and molecular characterization of new variants of SERPINA1 gene in Spanish patients with Alpha1-Antitrypsin Deficiency

N. Matamala<sup>1</sup>, D. Retana<sup>1</sup>, T. Fernandez<sup>1</sup>, A. Muñoz-Callejas<sup>1</sup>, B. Lara<sup>2</sup>, R. Sáez<sup>3</sup>, S. Castillo<sup>4</sup>, M. Molina<sup>5</sup>, A. Texido<sup>6</sup>, E. Lopez del amo<sup>7</sup>, G. Tirado<sup>8</sup>, J. Lopez-Campos<sup>9</sup>, I. Blanco<sup>10</sup>, B. Martinez-Delgado<sup>1</sup>;

<sup>1</sup>Institute of Health Carlos III (ISCIII), Madrid, Spain, <sup>2</sup>Royal Exeter & Devon Hospital, Exeter, Devon, United Kingdom, <sup>3</sup>Hospital Donostia, San Sebastián, Spain, <sup>4</sup>Hospital Clínico de Valencia, Valencia, Spain, <sup>5</sup>Bellvitge University Hospital-IDIBELL, Hospital de Llobregat, Spain, <sup>6</sup>Hospital Universitari Sant Joan de Reus, Tarragona, Spain, <sup>7</sup>Hospital La Laguna, Tenerife, Spain, <sup>8</sup>Hospital Cádiz, Cádiz, Spain, <sup>9</sup>Hospital Virgen del Rocío, Sevilla, Spain, <sup>10</sup>Alpha1-Antitrypsin Deficiency Spanish Registry (REDAAT), Spanish Society of Pneumology (SEPAR), Barcelona, Spain.

Alpha-1 antitrypsin deficiency (AATD) is caused by mutations in the SERPINA1 gene predisposing to early onset emphysema and liver cirrhosis. The most common mutations known to cause AATD are the deficient Z (Glu-342Lys) and S (Glu264Val) variants. Nevertheless, SERPINA1 gene is very polymorphic, and more than one hundred genetic variants have been described. We aimed to identify and characterize new variants of SERPINA1 gene in patients with decreased levels of serum AAT that cannot be explained by the genotype after analysis of Z and S alleles. We selected eight patients who fulfilled these criteria and performed sequence analysis of all coding exons of SERPINA1 by Sanger sequencing. We found seven previously unidentified missense variants, four of them located in exon 2, two in exon 3 and one in exon 4. Molecular characterization of these variants was performed by *in vitro* expression of mutant proteins generated after directed mutagenesis. We evaluated by western blot analysis the expression of monomeric AAT protein and the formation of polymers. In addition, protein aggregates were detected by Periodic acid-Schiff (PAS)-positive staining. Finally, an elastase assay was conducted in order to evaluate the inhibitory activity of the mutants. In conclusion, we have identified novel variants of SERPINA1 gene that can help to explain the discrepancies found between serum AAT levels and genotype. These results suggest that rare variants might be more frequent than expected, and therefore standardized PCR screening of the S and Z alleles should be complemented with sequencing of the gene in discordant cases.

## P13.07

### The coexistence of two causative mutations leads to reconsider Alport syndrome pattern of inheritance

C. Fallerini<sup>1</sup>, M. Baldassari<sup>1,2</sup>, E. Frullanti<sup>1</sup>, M. Mencarelli<sup>1,2</sup>, A. La Manna<sup>3</sup>, G. Garosi<sup>4</sup>, D. Del Prete<sup>5</sup>, A. Pinto<sup>1,2</sup>, F. Ariani<sup>1,2</sup>, F. Mari<sup>1,2</sup>, A. Renieri<sup>1,2</sup>;

<sup>1</sup>Medical Genetics, University of Siena, Siena, Italy, <sup>2</sup>Genetica Medica, Azienda

Ospedaliera Universitaria Senese, Siena, Italy, <sup>3</sup>Department of Pediatrics, Second University of Napoli, Napoli, Italy, <sup>4</sup>Nephrology, Dialysis and Transplantation, Azienda Ospedaliera Universitaria Senese, Siena, Italy, <sup>5</sup>Division of Nephrology, Department of Medicine DIMED, Laboratory of Histomorphology and Molecular Biology of the Kidney, University of Padova, Siena, Italy.

Alport syndrome (ATS) is a clinically heterogeneous progressive nephropathy, characterized by extreme phenotypic and genetic variability. Monogenic inheritance model is well known and different patterns of inheritance have been previously recognized ranging from autosomal to X-linked models. We have recently reported digenic inheritance for the disease. Here, we go further and we describe a new series of cases with more complex mechanism of inheritance for ATS unmasked by the increased use of high throughput sequencing which keeps unveiling unexpected combinations of pathogenic mutations lying either on the same allele or on different genes. Families with X-linked inheritance and unusual severity in females harbor two pathogenic mutations on the same COL4A5 allele. Families with apparent dominant pattern of inheritance are rather due to a digenic transmission of two causative mutations inherited in cis and lying in the two collagen 4 genes linked on chromosome 2 (COL4A3/COL4A4). Moreover, two distinct mutations on the same two genes, inherited in trans, are responsible for ATS following an apparent autosomal recessive mode of inheritance and intermediate severity. A more complex inheritance is shown when an autosomal mutation is combined with an X-linked mutation. Overall, these results reveal that ATS transmission pattern overcomes the Mendelian inheritance, which can thus easily trick the clinical geneticist. This finding leads to reconsider the recurrence risk and opens new perspectives in the management of genetic and prenatal counseling for ATS.

## P13.08

### MALAT1: a long non-coding RNA involved in alternative splicing regulation and potentially implicated in multiple sclerosis

E. M. Paraboschi<sup>1</sup>, G. Cardamone<sup>2</sup>, V. Rimoldi<sup>2,3</sup>, D. Gemmati<sup>4</sup>, M. Spreafico<sup>5</sup>, S. Duga<sup>2,3</sup>, G. Soldà<sup>2,3</sup>, R. Asselta<sup>2,3</sup>;

<sup>1</sup>Università degli Studi di Milano, Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Milano, Italy, <sup>2</sup>Humanitas University, Department of Biomedical Sciences, Rozzano, Italy, <sup>3</sup>Humanitas Clinical and Research Center, Rozzano, Italy, <sup>4</sup>University of Ferrara, Center Haemostasis & Thrombosis, Department of Medical Sciences, Ferrara, Italy, <sup>5</sup>Azienda Ospedaliera della Provincia di Lecco, Alessandro Manzoni Hospital, Department of Transfusion Medicine and Hematology, Lecco, Italy.

**Introduction:** Multiple sclerosis (MS) is an autoimmune neurodegenerative disease, characterized by chronic inflammation, demyelination, and axonal damage. Accumulating evidence suggests the existence of pathogenic links between MS and abnormalities in alternative splicing (AS). Among splicing regulators, besides hnRNPs and SR proteins, long noncoding RNAs (lncRNAs) are emerging as critical players in pre-mRNA AS. Here, we aimed at exploring the role of MALAT1, an abundant lncRNA reported to influence AS through its interaction with pre-mRNA splicing factors.

**Materials and Methods:** The expression levels of several splicing regulators, as well as the AS pattern of specific MS-related genes, were evaluated in HEK293, HeLa, and SH-SY5Y cells after MALAT1 overexpression or downregulation. Gene expression analysis was performed by real-time RT-PCR and RNAseq analysis.

**Results:** Upon MALAT1 modulation, expression levels of splicing regulatory genes, such as HNRNPF and CELF1, were significantly dysregulated in a cell-specific manner. Furthermore, we demonstrated that MALAT1 overexpression/silencing influence also the AS pattern of IL7R and PRKCA genes, previously associated with MS susceptibility and characterized by the presence of a SNP acting on splicing. Intriguingly, we also detected a significant upregulation (1.4-fold) of MALAT1 in peripheral blood mononuclear cells of MS patients respect to controls ( $P < 0.05$ ).

**Conclusion:** In this work, we provide evidence of a functional link between the lncRNA MALAT1, AS regulation and MS-associated AS events, suggesting a potential role of MALAT1 in MS pathogenesis.

## P13.09

### Expression Assay of BMPR2 gene in Pulmonary Arterial Hypertension

G. Pousada<sup>1</sup>, M. Lago<sup>1</sup>, S. Prado-López<sup>1</sup>, A. Baloira<sup>2</sup>, R. Varela-Calviño<sup>3</sup>, D. Valverde<sup>1</sup>;

<sup>1</sup>Universidad de Vigo, Vigo, Spain, <sup>2</sup>Complejo Hospitalario de Pontevedra, Pontevedra, Spain, <sup>3</sup>Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

Pulmonary Arterial Hypertension (OMIM 178600) is rare disease characterized by pulmonary vascular resistance increase and vascular remodelling. Symptoms include fatigue, shortness of breath and syncope. The most implicated gene is BMPR2, with a reduced expression in PASMC cells. The aim was to analyze the BMPR2 gene expression, in different cell cycle phases, in B-Lymphocytes.

We select seven patients with mutations and eight controls without mu-

tations in BMPR2. B-lymphocytes from patients and controls were isolated from peripheral blood by Ficoll method. B cells were immortalized using Epstein-Barr Virus and grown. Lymphoblastoid Cell line were analyzed by flow cytometry and separated based on the cell cycle phase by Sorting. With patients and controls RNA, we realized a RT-PCR and we analyzed BMPR2 gene expression with TaqMan assay.

After analysis of BMPR2 gene expression by qPCR, we detected expression for BMPR2 gene in Lymphoblastoid Cell line from controls. The normalized gene expression results in BMPR2 gene, using GADPH as reference gene, shows a greater expression levels in G2/M phase, followed by G0/G1 phase and, finally, in S phase. However, sorting cell analysis show changes in ploidy from patients relative to controls. The results of BMPR2 gene expression in patients, will tell us if there are any expression differences between patients and controls.

Although the availability of a tissue that express BMPR2 like lung is a problem, the expression analysis of the mutated gene by this technique, allowed us to analyzed how mutations in BMPR2 could interfere in the expression levels of this protein.

#### P13.10

#### WGS contribution to two cases of complex chromosomal rearrangements suggesting a chromothripsis mechanism

*N. Chatron<sup>1,2</sup>, J. Lauer<sup>1</sup>, P. Rollat Farnier<sup>1</sup>, A. Labalme<sup>1</sup>, F. Prieur<sup>3</sup>, F. Diguet<sup>1</sup>, M. Till<sup>1</sup>, R. Touraine<sup>2</sup>, C. Bardel<sup>1,4</sup>, P. Roy<sup>1,4</sup>, C. Schluth-Bördel<sup>1,2</sup>, D. Sanlaville<sup>1,2</sup>,*

<sup>1</sup>HCL, BRON Cedex, France, <sup>2</sup>INSERM U1028 CNRS UMR 5292, UCBL, CRNL, GENDEV Team, Lyon, France, <sup>3</sup>CHU Saint-Etienne, Saint-Etienne, France, <sup>4</sup>UMR 5558, LBBE, Service de Biostatistique et plateforme de séquençage NGS, CNRS, Lyon, France.

Chromothripsis was first described in 2011 in somatic cells and is proposed to constitute the basis for complex chromosomal rearrangements (CCR) seen in 2-3% of all cancers. These CCR were rarely reported in constitutional field. Here, we report CCR discovered in two patients harboring syndromic intellectual disability. The first case is a young boy with growth retardation, moderate intellectual disability, delayed speech, oral dyspraxia, hair ichthyosis, thin nails and facial dysmorphism. Karyotyping showed a ring 21 chromosome with 8 CNVs: 3 losses of 1.2 to 7.4 Mb and 5 gains of 162 kb to 2.8 Mb revealed by array CGH (aCGH) and confirmed by FISH. To reach basepair resolution of these anomalies, paired-end whole genome sequencing (WGS) (2x100bp) was performed on a NextSeq500 (Illumina). WGS confirmed all CNVs previously identified and pointed out three additional small CNVs (2 losses, 1 gain) and the presence of 4 inversions for a total of 23 chromosomal breakpoints. The second patient is an adult with mild intellectual disability, language delay and cleft palate carrier on the karyotype of a double chromosome 3 inversion (46,XX, inv(3)(p13; p22), inv(3)(p12; q26.3)) without pathogenic imbalance identified using aCGH. WGS revealed a more complex rearrangement with 12 breakpoints. The disruption of FOXP1 gene can explain Patient 2's phenotype. Overall, while Patient 1's phenotype is due to a large copy number change (losses and gains), the second case shows the importance of breakpoints' characterization of balanced chromosomal rearrangement and the potential underestimation of CCRs incidence

#### P13.11

#### Does the genomic architecture amongst Irish Travellers predispose to chromosome 17q12 microduplication?

*S. A. Lynch, J. McDaid, D. R. Betts, E. Crushell, J. P. Casey;  
National Centre for Medical Genetics, Dublin 12, Ireland.*

Certain regions of the human genome are predisposed to non-allelic homologous recombination (NAHR) resulting in copy-number-variants (CNVs). Ethnic differences have been observed with some CNVs. We previously reported a consanguineous Irish Traveller family with PCD and laterality due to homozygous mutations in CCDC103 (17q21.31). The mother (patient-A) and affected children had a co-existing chromosome 17q12 duplication. The 17q12 duplication and CCDC103 mutation are in cis, ~6Mb apart. Patient-A's husband (her first cousin) does not carry the 17q12 duplication indicating that a) this was a recent evolutionary CNV event either in patient-A or her mother (husband's maternal aunt) or b) there has been a recent recombination event.

We now report a second Irish Traveller family with a 17q12 duplication, identical to the first. This child (who has autism) also has a de novo neurexin-1 deletion (2q13.1). The families are not related and come from different geographical areas. Further analysis revealed that family-2 do not carry the CCDC103 mutation suggesting that a) the duplication arose as an independent de novo event in family 2 or b) recombination has separated the CCDC103 mutation from the duplication in previous generations. A review of 17q12 duplications identified within our centre revealed only three other cases, all in non-Traveller families.

Conclusions: The Irish Traveller population number ~40,000, out of a total population of 4.6 million, within the Irish Republic. Our discovery of two unrelated Irish Traveller families with a 17q12 duplication suggests that the genomic architecture at 17q12 in this population may predispose to this CNV.

#### P13.12

#### A comprehensive genome analysis of three constitutional chromothripsis rearrangements: a new mechanism for complex karyotype disorders

*A. Provenzano<sup>1</sup>, V. Orlandini<sup>1</sup>, M. Pantaleo<sup>2</sup>, G. Traficante<sup>1</sup>, L. Xumerle<sup>3</sup>, F. di Leva<sup>4</sup>, G. Bacci<sup>5</sup>, S. Stagi<sup>6</sup>, S. Guarducci<sup>7</sup>, S. Bargiaccchi<sup>1</sup>, O. Zuffardi<sup>7</sup>, M. Delle Donne<sup>3,4</sup>, S. Gigliot<sup>1</sup>,*

<sup>1</sup>Medical Genetics Unit, Department of Biomedical Experimental and Clinical Sciences „Mario Serio“, University of Florence, Firenze, Italy, <sup>2</sup>Medical Genetics Unit, Meyer Children's University Hospital, Firenze, Italy, <sup>3</sup>Personal Genomics srl, Verona, Italy, <sup>4</sup>Department of Biotechnologies, University of Verona, Verona, Italy, <sup>5</sup>Ophthalmology Unit, Meyer Children's University Hospital, Firenze, Italy, <sup>6</sup>Department of Health Science, University of Florence, Firenze, Italy, <sup>7</sup>Department of Molecular Medicine, University of Pavia, Pavia, Italy.

Chromothripsis is a novel phenomenon in the structural variation landscape of cancer genomes. We analyzed the genomes of three patients with congenital disease without apparent karyotype anomalies. The rearrangements displayed unanticipated complexity resembling chromothripsis. The array-CGH analysis identified complex chromosomal rearrangements involving chromosome 17 (Case-1), 12 (Case-2) and 1 and 4 (Case-3). Case-1 presented a complex clinical phenotype in which coexisted 3 known disorders: Potocki-Lupski syndrome, Charcot-Marie-Tooth disease and neurofibromatosis type 1. Case-2 showed intellectual disabilities with autistic traits and a particular stromal corneal dystrophy, at the moment described only in adult subjects. Case-3 exhibited a complex clinical picture, not immediately framed with 1p36 deletion syndrome (array-CGH revealed a 3 Mb deletion in 1p36). With whole genome sequencing we investigated the genetic architecture of these constitutional complex chromosomal rearrangements (CCRs), copy number profiling, and breakpoint-junctions proving that the rearrangements (deletions/duplications/inversions) identified in our cases were not clustered on a single chromosome region, but on the whole length of the chromosomes. These findings provide additional information suggesting that chromosome shattering and nonhomologous repair may be a common mechanism underlying chromothripsis rearrangements associated with developmental malformations.

#### P13.14

#### Short read paired end and long range mate pair genome sequencing characterization of complex and chromothriptic chromosomal aberrations

*D. Nilsson<sup>1</sup>, J. Eisfeld<sup>1</sup>, M. Pettersson<sup>1</sup>, F. Vezzi<sup>2</sup>, S. Lundin<sup>3</sup>, V. Wirta<sup>3</sup>, M. Käller<sup>3</sup>, M. Nordenskjöld<sup>1</sup>, E. Syk Lundberg<sup>1</sup>, A. Lindstrand<sup>1</sup>,*

<sup>1</sup>Karolinska institutet, Solna, Sweden, <sup>2</sup>SciLifeLab, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, <sup>3</sup>SciLifeLab, KTH Royal Institute of Technology, Stockholm, Sweden.

Complex chromosomal rearrangements, including chromothripsis, are emerging as important phenomena in both somatic and constitutional disease. Whole genome sequencing technologies capable of base pair level resolution of chromosomal fusion events, rearrangements and copy number changes now allow us to understand complex aberrations in fine detail. We present results from five complex cases using a combination of Illumina Nextera mate pair sequencing and PCR-free paired end (PE) sequencing. Two are cases of intrachromosomal chromothripsis affecting chromosome 1 and chromosome 21 respectively. The chromothriptic chromosome 21 was identified in a patient with heart anomalies and dysmorphic features, deceased at 5 mo. Molecular cytogenetic characterization with customized array comparative hybridization (CGH) and fluorescence in situ hybridization (FISH) had previously identified four deletions and five duplications. Whole genome sequencing elucidated break and fusion events at basepair resolution. BioNano Genomics IRYS nano-channel single molecule mapping was applied for corroboration. CNVs were estimated in excellent concordance with CGH using low pass short read coverage data, but at higher resolution, corroborating uncertain single probe signals. Discordant read pair data from low pass mate pair sequencing was sufficient to give a clear overall picture, improving over FISH reactions in all five cases. The added full coverage paired-end sequence data improves fine detail of complex rearrangements in fusion points. SNVs further add information with runs of autozygosity in deletions, and fractional allele ratios as expected from duplications. In summary, genome sequencing technologies elucidate even highly complex chromosome rearrangements, reducing analysis time, labour and increasing resolution over hybridisation based methods.



## P13.15

### Whole genome, mate-pair sequencing reveals additional cryptic breakpoints in a family with a complex chromosomal rearrangement

C. Aristidou<sup>1,2</sup>, M. Mehrjouy<sup>3</sup>, M. Bak<sup>3</sup>, N. Tommerup<sup>3</sup>, C. Sismani<sup>1,2</sup>;

<sup>1</sup>Cytogenetics and Genomics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>The Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>3</sup>Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark.

Accurate characterization of complex chromosomal rearrangements (CCRs) in non-affected individuals is crucial as their presence may result in reproductive failure, recurrent miscarriages or children with malformations. Here, we present a family, initially referred 10 years ago, where the non-affected father and daughter were found, using FISH and karyotyping, to be carriers of a balanced three-way translocation [t(6;7;10)(q16.2;q34;q26.1), de novo in the father]. The family suffered from two stillbirths, one miscarriage, and has a son with severe intellectual disability.

In the present study, the family was revisited using whole-genome mate-pair sequencing (MPS) and Sanger sequencing to further characterize the CCR in the father and daughter.

MPS allowed accurate reconstruction of all derivative chromosomes involved in the translocation. Interestingly, it revealed an additional cryptic translocation breakpoint on der(6) (1.37Mb proximal to the 1st breakpoint) rendering the rearrangement even more complex. The interstitial segment created was translocated onto der(10) proximal to the translocated segment from chromosome 7.

SIM1, GRIK2, CNTNAP2, and PTPRE genes were disrupted at the breakpoints. As some of these genes have been proposed as candidate dominant genes for developmental delay, clarifying the consequences of the disruption at the protein level will provide important information about the function of the genes.

In conclusion, MPS proved highly successful in identifying additional complexity, not detectable by FISH, in two non-affected complex translocation carriers. We propose that such CCRs should be studied with NGS in combination with other methods to aid in correct prenatal, preimplantation genetic diagnosis and counselling in couples with reproductive problems.

## P13.16

### Complex deletion of 18q revealed by array CGH - the significance of high resolution analysis method in patients with known abnormal karyotype

M. Czakó, K. Hadzsiev, A. Szabó, A. Lőcsei-Fekete, B. Melegh;

University of Pécs, Department of Medical Genetics, Pécs, Hungary.

Introduction: Inverted duplications with terminal deletions have been described with different chromosomes involved. Since the widespread use of array CGH increasing number of publications revealed the possible mechanisms of its development as non-allelic homologous recombination or nonhomologous end joining resulting in a dicentric chromosome, in which a breakage-fusion-bridge cycle and telomere formation lead to this type of rearrangement. In the vast majority of the known cases the duplicated region is larger than the deleted segment. In the patient presented here the situation is exactly the opposite.

Methods: Routine cytogenetic investigation was indicated in a 5 year old child because of muscle hypotonia, clubfoot, genital hypoplasia, minor anomalies and delayed development. The deletion of terminal 18q detected by karyotyping (450 bands), however, didn't provide sufficient explanation for the severity of the symptoms, therefore array CGH analysis using Agilent Sureprint 8x60K oligo-array (ISCA v.2) was performed.

Results: Array CGH detected a 2,166 Mb duplication affecting 18q21.31q21.32 and a 21.5 Mb contiguous deletion of 18q21.32q23. In possession of the results the more complex clinical features compared to "pure" deletion 18q syndrome could be explained, allowing for a more thorough genotype-phenotype analysis.

Conclusions: The application of high-resolution molecular cytogenetic analysis methods is of great importance even in cases where the karyotyping detects abnormalities which correlation with the clinical picture is clear. In order to understand the formation of chromosomal rearrangements better, the use of array CGH analysis providing copy number data for the regions adjacent to the breakpoints is recommended.

## P13.17

### Conserved non-coding elements and Topologically Associated Domains, a correlation story

B. Ambroise, J. C. Fischer, L. E. Hunt, G. Elgar;

The Francis Crick Institute, London, United Kingdom.

The recent development of genome wide chromosome conformation capture (Hi-C) has permitted the study of chromatin interactions within the nucleus. The resulting interactions maps show that that genomes can be divided into large local chromatin domains termed Topologically Associated Domains (TADs). Within TADs, the genome appears to be organized to favour strong internal chromatin interactions rather than external interactions with neighbouring TADs. It has also been suggested that TADs might help to delineate basic genomic functions such as gene regulation. Interestingly, TADs also appear to be conserved across species and cell types.

Comparative genomics has revealed the existence of conserved non-coding elements (CNEs) in vertebrates. Multiple lines of evidence suggest that these non-coding units are cis-regulatory elements associated with key developmental genes.

Using CNEs clusters extracted from the CONDOR database, we investigated whether CNEs and TADs were spatially correlated in the human genome. We looked at different parameters such as absolute and relative distances, overlap of the regions and correlation of the boundaries. In addition, we performed 10,000 simulations to measure the statistical robustness of our analysis.

Preliminary results show a strong correlation between the boundaries of CNE clusters and TADs. This indicates that CNEs are not randomly distributed across the genome but are organized with respect to the TAD architecture. Furthermore, this reinforces the role of CNEs as potential cis-regulatory modules of genes.

## P13.18

### A case of potential human chimerism detected by the concomitant presence of both derivative chromosomes from the same translocation.

M. E. Mansilla<sup>1,2</sup>, F. García-Santiago<sup>1,2</sup>, M. A. Mori<sup>1,2</sup>, S. García-Miñaur<sup>1,2</sup>, J. Nevado<sup>1,2</sup>, L. Fernández<sup>1,2</sup>, B. Fernández<sup>1,2</sup>, M. I. Gómez<sup>1,2</sup>, P. Lapunzina<sup>1,2</sup>;

<sup>1</sup>INGEMM, IIdiPaz. Hospital Universitario la Paz, Madrid, Spain, <sup>2</sup>CIBERER. Instituto de Salud Carlos III, Madrid, Spain.

A complex genetic diagnosis of a 2-year-old girl with growth delay, congenital renal anomalies, and developmental delay was revealed by combined a-CGH and FISH studies. The reported girl was born single to healthy non-consanguineous parents.

Methods and results: oligonucleotide-based microarray analysis was performed on genomic DNA (KaryoArray®, Bluegnome, Illumina). A mosaicism of a 8,84 Mb terminal deletion on 10q26 and a 511 Kb terminal duplication on 18q initially suggested an unbalanced derivative chromosome 10. Subtelomeric 10q (Vysis) and subtelomeric 18q (Kreatech) FISH probes were applied to establish mosaicism degree, resulting in a major cell line (171 metaphases) with the derivative chromosome 10 (one signal for subtelomeric 10q region and three signals for subtelomeric 18q region) and a minor one (8 metaphases) with the derivative chromosome 18 (three signals for subtelomeric 10q region and one signal subtelomeric for 18q region). No metaphases with the balanced translocation was detected. Parents' karyotype and FISH studies were normal.

Conclusions: The mosaicism observed by a-CGH is the result of the dosage compensation between the two chromosome derivative. The FISH techniques are essential for the full characterization of genomic rearrangements. We discuss the possible genetic mechanism involving the occurrence of at two independent events: germinal mosaicism and human chimerism in a single pregnancy after an initial twin dizygotic conception

## P13.19

### Highly restricted Down syndrome critical region identified on human chromosome 21

M. C. Pelleri<sup>1</sup>, E. Cicchini<sup>2</sup>, C. Locatelli<sup>2</sup>, L. Vitale<sup>1</sup>, M. Caracausi<sup>1</sup>, A. Piovesan<sup>1</sup>, A. Rocca<sup>2</sup>, G. Poletti<sup>2</sup>, M. Seri<sup>3</sup>, P. Strippoli<sup>1</sup>, G. Cocchi<sup>4</sup>;

<sup>1</sup>Department of Experimental, Diagnostic and Specialty Medicine (DIME), Unit of Histology, Embryology, Bologna, Italy, <sup>2</sup>Neonatology Unit, St. Orsola-Malpighi Polyclinic, Bologna, Italy, <sup>3</sup>Medical Genetics Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy,

<sup>4</sup>Neonatology Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy.

Introduction: A „Down Syndrome critical region“ (DSCR) sufficient to induce the most constant phenotypes of Down syndrome (DS) had been identified by studying partial (segmental) trisomy 21 (PT21) as an interval of 0.6-8.3 Mb within human chromosome 21 (Hsa21), although its existence was later

questioned.

**Materials and Methods:** We propose an innovative, systematic reanalysis of all described PT21 cases (from 1973 to 2015). In particular, we build an integrated, comparative map from 126 cases with or without DS fulfilling stringent cytogenetic and clinical criteria. The map allowed to define or exclude as candidates for DS fine Hsa21 sequence intervals, also integrating duplication copy number variants (CNVs) data.

**Results:** A highly restricted DSCR (HR-DSCR) of only 34 kb on distal 21q22.13 has been identified as the minimal region whose duplication is shared by all DS subjects and is absent in all non-DS subjects. Also being spared by any duplication CNV in healthy subjects, HR-DSCR represents a strong candidate for the typical DS features, the intellectual disability and some facial phenotypes. HR-DSCR contains no known gene and has homology only to the chimpanzee genome. Conclusion: Our results strongly support the view that a single main critical region for DS actually exists, that it appears to be much smaller than previously suspected and that it could contain currently undescribed genes whose identification should become a priority for understanding the fundamental genotype-phenotype relationships in DS and in searching for highly relevant targets for a cure of DS.

#### P13.20

##### First report of MEFV duplication in FMF patient

*G. Sarrabay<sup>1</sup>, D. Mechin<sup>2</sup>, B. Dumont<sup>1</sup>, M. Andre<sup>2</sup>, I. Touitou<sup>1</sup>;*

*<sup>1</sup>UMAI laboratoire de génétique, Montpellier, France, <sup>2</sup>Médecine Interne CHU Clermont Ferrand, Clermont Ferrand, France.*

Familial Mediterranean fever (FMF) is a rare monogenic disease and the prototype of autoinflammatory disorders. It is caused by mutations in the MEFV gene and is autosomally recessively inherited. Most mutations are missense substitutions, small deletions are quite rare, and only three nonsense mutations have been described. Large rearrangements have been searched for in the frame of a collaborative project including 216 patients but were not identified.

We report here a 21 years-old woman who presented with classical FMF phenotype: recurrent fever, arthralgia, and abdominal pain with vomiting. Attacks lasted three days and biological inflammation was documented with elevated C-reactive protein. Her father is Armenian and her mother Malagasy, and both are asymptomatic.

We identified a well-known severe mutation: p.Met694Val, and a controversial variant: p.Glu148Gln. Parental testing confirmed that the variants were non-allelic. Sanger sequencing displayed unbalanced ratio of the mutated and wild type alleles. Mosaicism was excluded because all polymorphisms were found at the same 1:2 ratio. DNA contamination was ruled out through microsatellite analysis. We thus suspected a gene micro-rearrangement. Quantitative polymerase chain reaction and deep-sequencing revealed a heterozygous duplication of the entire wild MEFV gene inherited from the mother. Two close surrounding genes (NAA60 and OR1F1) were not duplicated demonstrating that this rearrangement was confined to the MEFV region.

We report here the first MEFV duplication in a FMF patient who subsequently expressed 1/3 dose of p.Met694Val. Interestingly, the "dilution" of the pathological variants did not moderate the patient's phenotype.

#### P13.21

##### Functional assays and bioinformatics predictions reveal a high contribution of splicing mutations in the most frequent forms of hereditary cancer

*O. Soukarieh<sup>1,2</sup>, P. Gaildrat<sup>1,2</sup>, G. Castelain<sup>1,2</sup>, S. Krieger<sup>1,2,3</sup>, S. Baert-Desurmont<sup>1,2,4</sup>, D. Di Giacomo<sup>1,2</sup>, J. Abdat<sup>1,2</sup>, H. Tubeuf<sup>1,2</sup>, A. Kilian<sup>1,2</sup>, J. Thery<sup>1,2</sup>, C. Bonnet<sup>1,2</sup>, S. Akli<sup>1,2</sup>, A. Drouet<sup>1,2</sup>, M. Vezain<sup>1,2</sup>, M. Hamieh<sup>1,2</sup>, E. Rouleau<sup>5</sup>, I. Tournier<sup>1,2</sup>, S. Caputo<sup>5</sup>, C. Houdayer<sup>5,6</sup>, T. Frébourg<sup>1,2,4</sup>, M. Tosi<sup>1,2</sup>, A. Martins<sup>1,2</sup>, in collaboration with the French Oncogenetics Network;*

*<sup>1</sup>Inserm U1079, University of Rouen, Institute for Research and Innovation in Biomedicine, Rouen, France, <sup>2</sup>Normandy Centre for Medical Genomics and Personalized Medicine, Rouen, France, <sup>3</sup>Department of Clinical Biology and Oncology, François Baclesse Cancer Centre, Caen, France, <sup>4</sup>Department of Genetics, University Hospital, Rouen, France, <sup>5</sup>Genetics Department, Curie Institute, Paris, France, <sup>6</sup>University Paris Descartes, Paris, France.*

The identification of a causal mutation is essential for molecular diagnosis and clinical management of hereditary cancers. However, even if high-throughput DNA sequencing has greatly improved the detection of nucleotide changes, the biological interpretation of most variants remains challenging. Here, we report the development and outcome of minigene assays allowing the identification of splicing mutations in any gene of interest. We analyzed in this system more than 600 variants identified in genes implicated in Lynch syndrome or in hereditary breast and ovarian cancer syndrome, notably MLH1, MSH2, BRCA1 and BRCA2. Patient RNA was also analyzed

when available. Our results indicate that more than 25% of variants of unknown significance in these genes have an impact on RNA splicing, an information that contributed to the clinical classification of several of these variants. Moreover, our targeted studies on "model-exons", including MLH1 exon 10 and BRCA2 exon 7, revealed an unexpected large number of variants altering potential exonic splicing regulatory elements (ESR), an effect that could not be predicted by commonly used bioinformatics approaches. We then evaluated the predictive power of three newly developed ESR-dedicated algorithms. Our results pinpointed the good sensibility and specificity of two of these methods both at identifying ESR mutations and at predicting their severity. This study highlights the potential of *in silico* approaches as filtering tools for prioritizing variants for functional analyses, a strategy that may help identifying pathogenic variants among the plethora of nucleotide changes detected by exome sequencing. Our findings have implications for all genetic disorders.

#### P13.22

##### Detection of genomic alterations using high-throughput technology in Brazilian patients with hearing loss

*P. L. Jacob<sup>1</sup>, S. M. Da Silva Costa<sup>1</sup>, G. O. L. Rodrigues<sup>2</sup>, J. A. Yunes<sup>2</sup>, A. M. Castilho<sup>3</sup>, E. L. Sartorato<sup>4</sup>;*

*<sup>1</sup>Human Molecular Genetics Laboratory, Molecular Biology and Genetic Engineering Center (CBMEG) - University of Campinas, Campinas, Brazil, <sup>2</sup>Children's Hematological Investigations Center Dr. Domingos Boldrini, Campinas, Brazil, <sup>3</sup>Otorhinolaryngology Department, University of Campinas (UNICAMP), Campinas, Brazil.*

Hearing loss is one of the most common sensory disorders in humans. The identification of genetic variants associated with deafness can contribute to a better understanding of the molecular basis and the pathophysiological mechanisms involved in the different phenotypes of hereditary hearing loss. Moreover, it can provide an accurate diagnosis, development of specific treatments and genetic counseling for patients and families. The application of new high-throughput technologies, such as CytoScan HD Array, allows an optimization of genetic diagnosis and a comprehensive investigation of hereditary hearing loss. The main goal of the present study was to elucidate the genetic etiology in a cohort of Brazilian patients heterozygous for GJB2 gene mutation with hearing loss using the CytoScan HD Array Microarray. Genomic DNA from 14 patients with hearing loss was evaluated by CytoScan HD Array (Affymetrix). We found 68 genomic changes. The most significant changes were observed on chromosome 13 (13q12.11), near the GJB6 gene (Connexin 30). It was observed a loss of heterozygosity (LOH) in three regions. The first interval have 419,27kb of size, where are located the genes PSPCI, ZMYMS, ZMYM2, GJA3; in an intronic region there is an interval with 179,84kb of size, and other interval with 348,69kb which includes the genes CRYL1, IFT88, MIR 4499. To date, we are mapping the flaking regions of these intervals in order to study the segregation of these changes in the other family members. These quantitative changes may play a role in the development of hereditary hearing loss.

#### P13.23

##### Functional analysis of an Ay-globin gene promoter variant (HBG1: g.-225\_-222delAGCA) underlines its role in increasing fetal hemoglobin levels under erythropoietic stress

*J. D. Vasiljevic<sup>1</sup>, M. M. Ugrin<sup>1</sup>, M. M. Stojiljkovic<sup>1</sup>, B. Zukic<sup>1</sup>, K. Klaassen<sup>1</sup>, T. Katsila<sup>2</sup>, L. Dokmanovic<sup>3</sup>, D. Janic<sup>3</sup>, G. P. Patrinos<sup>2</sup>, S. Pavlovic<sup>1</sup>;*

*<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Department of Pharmacy, University of Patras School of Health Sciences, Patras, Greece, <sup>3</sup>Department of Hematology and Oncology, University Children's Hospital, School of Medicine, University of Belgrade, Belgrade, Serbia.*

Heredity persistence of fetal hemoglobin is a condition characterized by persistent  $\gamma$ -globin gene expression and synthesis of high levels of fetal hemoglobin (HbF;  $\alpha 2\gamma 2$ ) during adult life. It is usually caused by promoter variants affecting the human fetal globin genes (HBG1 and HBG2). Some of these variants, such as HBG2: g.-158C>T, exert their effect only under conditions of erythropoietic stress, typical for  $\beta$ -thalassemia patients. We analyzed a previously reported deletion residing in the promoter region of the HBG1 gene (HBG1: g.-225\_-222delAGCA), both in normal conditions and under conditions of erythropoietic stress. Our results indicate that this deletion is responsible for decreased HBG1 gene expression. Specifically, this deletion was shown to result in drastically reduced reporter gene expression in K562 cells, compared to the wild-type sequence but only under conditions of erythropoietic stress, mimicked by introduction of erythropoietin into the cell culture. Also, electrophoretic mobility shift analysis showed that the HBG1: g.-225\_-222delAGCA deletion creates additional transcriptional factors' binding sites, which, we propose, bind a transcriptional repressor, thus decreasing the HBG1 gene promoter activity. These results are consistent

with *in silico* analysis, which indicated that this deletion creates a binding site for GATA1, known to be a repressor of the  $\gamma$ -globin gene expression. These data confirm the regulatory role of the *HBG1*: g.-225\_-222 region that exerts its effect under conditions of erythropoietic stress characteristic for  $\beta$ -thalassemia patients.

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#### P13.24

#### Identification of binding partners of the LPAR6 protein involved in hypotrichosis

**M. T. Romano<sup>1</sup>, S. M. Pasternack-Ziach<sup>1</sup>, B. Basmanav<sup>1</sup>, S. Kittanakom<sup>2</sup>, A. M. Oprisoreanu<sup>3</sup>, N. Ishorst<sup>1</sup>, A. Linneborn<sup>1</sup>, S. Schöch<sup>3</sup>, I. Stagljar<sup>2</sup>, R. C. Betz<sup>1</sup>;**

<sup>1</sup>*Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Biochemistry, University of Toronto, Toronto, ON, Canada, <sup>3</sup>Institute of Neuropathology, University of Bonn, Bonn, Germany.*

**Introduction:** Hypotrichosis simplex (HS) is a genetically heterogeneous hair loss disorder characterized by progressive hair loss. Mutations in more than 10 genes have been identified for HS, including the LPAR6 gene encoding for the human lysophosphatidic acid receptor 6 (LPAR6). This protein belongs to the same signaling network of lipase H (LIPH), which is also a causative gene for HS. Considering the importance of this pathway in the determination of proper hair growth, we speculated that more genes related to the same pathway could be a cause of HS. Therefore, our goal was to identify new interactors of LPAR6.

**Materials and methods:** Membrane yeast two hybrid (MYTH) assay and bait-dependency test were performed to spot putative binding partners (BPs) of this receptor. The results revealed 72 putative interactors from the skin/hair follicle library. The last step was to further assess the interaction by performing pull-down assay, to discard false positive results and, at the same time, confirm the interaction between LPAR6 and the candidate proteins.

**Results:** Using this methodology, five true BPs have already been confirmed, including the U11/U12 small nuclear ribonucleoprotein 35 kDa protein (SNRNP35), the 40S ribosomal protein S28 (RPS28), the emopamil-binding protein (EBP), the transcription factor AP-1 (JUN) and the lymphocyte antigen 6 complex locus D (LY6D).

**Conclusions:** We were able to identify some of the BPs of LPAR6. Many more putative interactors have been already been cloned and await to be studied.

#### P13.25

#### Mechanistic Insight into Formation of Chromosomal Insertions

**P. Stankiewicz<sup>1,2</sup>, S. Gu<sup>1</sup>, P. Szafranski<sup>1</sup>, Z. H. C. Akdemir<sup>3</sup>, B. Yuan<sup>1</sup>, M. A. Magrina<sup>3</sup>, C. A. Bacino<sup>1,2</sup>, S. L. Lalani<sup>1,2</sup>, A. M. Bremner<sup>1,2</sup>, J. L. Smith<sup>1,2</sup>, A. Patel<sup>1,2</sup>, S. W. Cheung<sup>1,2</sup>, C. M. B. Carvalho<sup>1</sup>, W. Bi<sup>1,2</sup>, J. R. Lupski<sup>1,4,5</sup>;**

<sup>1</sup>*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States, <sup>2</sup>Baylor Miraca Genetics Laboratories, Houston, TX, United States,*

<sup>3</sup>*Instituto Hermes Pardini S.A., São Paulo, Brazil, <sup>4</sup>Department of Pediatrics, Baylor College of Medicine, Houston, TX, United States, <sup>5</sup>Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, United States.*

Chromosomal insertions are genomic rearrangements with a chromosome segment inserted into a non-homologous chromosome or a different region on the same chromosome. Insertions, usually revealed through G-banded chromosome or FISH analyses, constitute ~2% of nonrecurrent copy-number gains. Very little is known about the molecular mechanisms of their formation. Recently, microhomologies at the insertion sites were found in a few cases, suggesting a replicative mechanism of formation. We identified 16 individuals with complex insertions among 56,000 individuals tested at Baylor Miraca Genetics Laboratories using FISH and clinical array comparative genomic hybridization (aCGH). Custom high-density aCGH was performed on 10 individuals with available DNA, and breakpoint junctions were fine-mapped at nucleotide resolution by long-range PCR and DNA sequencing in six individuals. We observed microhomologies and templated insertions at the breakpoint junctions, resembling the signatures found in CGRs and chromoanansynthesis involving two or three chromosomes generated through replication-based mechanism(s) such as FoSTeS/MMBIR. In addition, using custom high-density aCGH, we studied 11 families with unbalanced simple insertions in children, inherited from a parent, carrier of an apparently balanced insertion detected by FISH. We found that three parents had additional small CNVs at one or both sides of the inserting fragments. Moreover, in one family, we identified an additional submicroscopic reciprocal insertion. In conclusion, we propose that significant fraction of both complex insertions generated through chromoanansynthesis involving two or more chromosomes and apparently balanced simple insertions are caused by DNA replication with template switching errors.

#### P13.27

#### Differential effects of LINE-1 methylation level on chromosome segregation during pre- and postnatal human ontogenesis

**S. A. Vasilyev<sup>1,2</sup>, E. N. Tolmacheva<sup>1</sup>, A. V. Agab<sup>3</sup>, A. A. Kashevarova<sup>1,2</sup>, E. A. Sazhenova<sup>1</sup>, R. R. Savchenko<sup>2</sup>, T. V. Nikitina<sup>1</sup>, I. N. Lebedev<sup>1,2,3</sup>;**

<sup>1</sup>*Scientific Research Institute of Medical Genetics, Tomsk, Russian Federation, <sup>2</sup>Tomsk State University, Tomsk, Russian Federation, <sup>3</sup>Siberian State Medical University, Tomsk, Russian Federation.*

**Introduction:** Aneuploidy can lead to changes in epigenetic landscape and, vice versa, epigenetic errors can cause aneuploidy. In this study, a relationship between the level of DNA methylation and aneuploidy was analyzed in human cells during pre- and postnatal stages of ontogenesis.

**Materials and Methods:** Retrotransposon LINE-1 methylation was assessed by pyrosequencing in extraembryonic mesoderm and trophoblast of 17 full and 35 mosaic trisomic miscarriages, 21 induced abortions and lymphocytes of 24 healthy individuals. Spontaneous chromosome loss rate (SCLR) was analyzed in vitro by micronucleus test with FISH in extraembryonic fibroblasts and adult lymphocytes.

**Results:** LINE-1 methylation index was similar among pure trisomics (mostly of meiotic origin) and induced abortions (50-53%), but it was higher (55-56%, p=0.003) in miscarriages with mosaicism. Perhaps, this result from incomplete epigenetic genome reprogramming at preimplantation stage and may be accompanied by hypermethylation of checkpoint genes leading to aneuploidy. In adult lymphocytes, significantly higher LINE-1 methylation index (75%) was observed.

SCLR did not differ significantly between groups of embryos with different LINE-1 methylation index. However, SCLR was lower in adult lymphocytes (2.6±1.3%) than in fibroblasts of trisomic (5.5±5.8%, p=0.03) and induced (3.7±3.0%, p=0.09) abortions. One can suggest that this results from centromere conformation abnormalities due to genome hypomethylation in extraembryonic tissues.

**Conclusions:** LINE-1 hypomethylation in extraembryonic mesoderm is associated with mitotic SCLR in comparison with adult lymphocytes. In contrast, LINE-1 hypermethylation can lead to mosaicism in early embryo development.

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#### P13.29

#### Systematic investigation of a potential role of microRNAs in the pathogenesis of male pattern baldness

**L. M. Hochfeld<sup>1,2</sup>, T. Anholt<sup>1,2</sup>, A. Hofmann<sup>3</sup>, N. Fricker<sup>1,2</sup>, M. M. Nöthen<sup>1,2</sup>, S. Heilmann-Heimbach<sup>1,2</sup>;**

<sup>1</sup>*Institute of Human Genetics, University of Bonn, Bonn, Germany, <sup>2</sup>Department of Genomics, Life&Brain Center, University of Bonn, Bonn, Germany, <sup>3</sup>Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany.*

Male pattern baldness (MPB) is characterised by an androgen-dependent loss of hair from the frontal and vertex area of the scalp which does not affect hair follicles (HFs) of the occipital scalp area. So far, the biological mechanisms that underlie these paradox differences in androgen-sensitivity between HF-subpopulations remain elusive. As microRNAs have been demonstrated to be differentially expressed in androgen-dependent vs. androgen-independent tissues and have already been implicated in hair biology, we hypothesised, that microRNAs may contribute to the differences in androgen-sensitivity between HF-subpopulations during MPB-pathogenesis. We therefore performed microRNA-profiling and differential expression analysis in HFs from the frontal and occipital scalp of 25 healthy male donors. A total of 42 microRNAs showed significant differences in gene-expression between frontal and occipital HFs. To identify potential target genes and biological pathways of these microRNAs, we searched miRWalk2.0 for validated and predicted target genes. Expression of 104 validated and 956 predicted target genes in human HF was confirmed using an in-house data set on HF mRNA expression. The subsequent Ingenuity pathway-based analysis revealed an enrichment of HF-expressed target genes in mTOR- and AKT-signalling, which have been implicated in cell proliferation and HF-homeostasis and androgen- and IL-1-signalling, the latter being described to influence androgen-regulated gene expression in dermal papilla cells. In summary, our data render a role of microRNAs in MPB-development via the control of HF-sensitivity to androgens likely. Additional studies are now warranted to elucidate the exact molecular mechanisms and to evaluate microRNAs as potential new drug targets for MPB therapy.

## P13.30

**Profound developmental delay, congenital heart disease, epilepsy, hearing impairment and dysmorphisms in a one-year-old girl caused by a novel heterozygous de novo missense variant in MED13L**I. M. Bader<sup>1</sup>, C. Rauscher<sup>2</sup>, W. Sperl<sup>2</sup>, O. Rittinger<sup>1</sup>, T. B. Haack<sup>3,4</sup>, R. Kovács-Nagy<sup>2</sup>, T. Meitinger<sup>3,4</sup>;<sup>1</sup>Clinical Genetics Unit; Children's Hospital; Paracelsus Medical University, Salzburg, Austria, Salzburg, Austria, <sup>2</sup>Children's Hospital; Paracelsus Medical University, Salzburg, Austria, Salzburg, Austria, <sup>3</sup>Institute of Human Genetics, Technische Universität München, Munich, Germany, <sup>4</sup>Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany.

Heterozygous *MED13L* variants are reported to cause a spectrum of phenotypes ranging from isolated congenital heart disease to a syndromic form of intellectual disability (ID) described recently (Adegbola et al. 2015).

While missense mutations in *MED13L* have early been associated with the cyanotic form of non-syndromic congenital heart diseases including dextro-looped transposition of the great arteries (MIM#608880), a *MED13L* haploinsufficiency syndrome has recently been described in two patients with moderate ID, conotruncal heart defects, facial abnormalities and hypotonia (Asadollahi et al. 2013). Ten further patients with severe syndromic ID, delay in motor and speech development but without cardiac phenotypes have been reported, mainly associated with dosage changes

(van Heest et al., 2015, Adegbola et al. 2015). One report exists about a homozygous missense variant in *MED13L* in a patient with mild ID (Najmabadi et al. 2011).

Here we present the phenotype of a one-year-old girl with coarctation of the aorta, profound developmental delay, epilepsy, hearing impairment and with additional dysmorphisms that fit into the syndromic spectrum found in patients with *MED13L* haploinsufficiency.

Whole exome sequencing and molecular karyotyping revealed a novel *de novo* heterozygous missense mutation in *MED13L* as the likely cause of the condition in this girl.

The accumulation of clinical signs and symptoms, dysmorphisms as well as the severity of the profound developmental delay rises the hypothesis that the novel missense mutation in *MED13L* in this girl could exert a dominant negative effect.

## P13.31

**Identification of genes that escape X-inactivation**M. Rask-Andersen<sup>1,2</sup>, Å. Johansson<sup>2</sup>;<sup>1</sup>Uppsala University, Uppsala, Sweden, <sup>2</sup>Uppsala University, department of Immunology, genetics and pathology, Uppsala, Sweden.

Introduction: Cells of females contain two copies of the X chromosome. To avoid high expression of X-linked genes, due to gene dosage effects, one of the X-chromosomes are silenced by a mechanism called X-inactivation. This mechanism is associated with specific histone modifications and hypermethylation along the inactivated chromosome. However, a number of genes escape silencing by an unknown mechanism. In this study we utilize epigenome wide DNA methylation data to identify genes that escape X-inactivation and to contrast our findings with available public DNA methylation datasets from different tissues.

Methods: The study included 732 participants (389 female and 341 male). DNA methylation in blood samples was assayed using the Infinium Human-methylation450 BeadChip which includes 11232 probes on the X-chromosome. Escape from X-inactivation was defined as probes with an average methylation below 15% in both males and females; and non-overlapping methylation ranges.

Results: A total of, 579 CpG probes associated with 160 unique genes displayed DNA methylation patterns that were consistent with X-inactivation escape. This pattern was particularly enriched on the short arm of chromosome X, which is consistent with previous publications. The X-chromosome contains 827 genes and escape from X-inactivation was detected for approximately 19% of these genes.

Conclusion: DNA methylation data can be utilized to identify and study regions of the X-chromosome that escape inactivation. Further studies may reveal how X-inactivation and escape is related to complex traits and diseases, as well as X-inactivation dynamics related to age.

## P13.32

**Missense mutations leading to exon skipping in ATP7A**

L. B. Moeller;

Kennedy Center, Rigshospitalet, Glostrup, Denmark.

Menkes disease (MD) is caused by mutations in *ATP7A*, encoding a copper-transporting P-type ATPase. Missense mutations have been widely used to

identify functionally important amino acid residues, but it is necessary to be cautious. By investigation the molecular effect of 36 *ATP7A* missense mutations identified in phenotypic different MD patients, we found that 7 out of the 36 *ATP7A* missense mutations affect splicing, leading to absence of protein product due to nonsense mediated decay of the *ATP7A* transcript or to truncated forms of the ATPase due to exon skipping. Three of the mutations affects nucleotides located at the 3'end of exon 10, 12 and 15 respectively and lead to skipping of exon 10, 12 and 15 probably as a result of poor 3'donor splice site recognition. Four missense mutations, two located in the middle of exon 8 and two in the middle exon 21 lead to skipping of exon 8 and exon 21 respectively probably due to effects on exonic splicing silencers (ESS) or exonic splicing enhancers (ESE), as these mutations lead to increased ESS/ESE values. The exon skipping lead in some cases to in frame transcript and in other cases to premature termination codon due to frameshift. Normal amount of transcript was observed if the exon skipping preserves the reading frame or if the premature termination codon was located close to the last exon, exon 23.

## P13.34

**Identification of type-1 NF1 deletion breakpoints mediated by a novel mutational mechanism, palindrome-induced NAHR**A. Summerer<sup>1</sup>, M. Hillmer<sup>1</sup>, V. Mautner<sup>2</sup>, D. Cooper<sup>3</sup>, L. Messiaen<sup>4</sup>, H. Kehrer-Sawatzki<sup>1</sup>;<sup>1</sup>Institute of Human Genetics, University of Ulm, Ulm, Germany, <sup>2</sup>Department of Neurology, University Hospital Hamburg Eppendorf, Hamburg, Germany, <sup>3</sup>Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom,<sup>4</sup>Medical Genomics Laboratory, Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, United States.

Neurofibromatosis type 1 (NF1) occurs with an incidence of 1 in 3000. In 5% of all patients with NF1, the cause of the disease is a large deletion encompassing the NF1 gene and its flanking regions. The majority of these large NF1 deletions are type-1, encompassing 1.4-Mb with breakpoints located in the low-copy repeats NF1-REP<sub>A</sub> and NF1-REP<sub>B</sub> which exhibit high sequence homology over a 50-kb region. Previous studies have indicated that non-allelic homologous recombination (NAHR) constitutes the major mutational mechanism underlying type-1 NF1 deletions. In the present study, we analysed 118 patients with type-1 deletions by MLPA. In 99 (84%) of the 118 deletions, the breakpoints were located within the NAHR hotspots PRS1 and PRS2, separated by 20-kb. However, 19 deletions did not exhibit breakpoints located within PRS1 or PRS2, and the aim of this study was to identify and characterize these breakpoints. To this end, we performed high-resolution array CGH and analysed overlapping long-range PCR-products using paralog-specific primers. Our results indicate that many of these type-1 deletions not mediated by NAHR within the known NAHR hotspots exhibit breakpoints close to or within long palindromic sequences capable of forming hairpin or cruciform structures. These findings imply a novel mutational mechanism, termed palindrome-induced NAHR, which is triggered by a secondary structure-induced DNA double strand break (DSB). By contrast, type-1 NF1 deletions with breakpoints located within the NAHR hotspots are likely to be regulated by PRDM9 which binds to specific DNA sequence motifs and initiates recombination by recruiting the DSB-machinery.

## P13.35

**Non-coding variant in a remote and putative enhancer in the cis-regulatory domain of *FOXL2* found in a multigenerational Polynesian family with BPES**H. Verdin<sup>1</sup>, A. Shelling<sup>2</sup>, D. Markie<sup>3</sup>, A. L. Vincent<sup>4,5</sup>, E. De Baere<sup>1</sup>;<sup>1</sup>Center for Medical Genetics, Ghent University, Ghent, Belgium, <sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand, <sup>3</sup>Pathology Department, Dunedin School of Medicine, Otago University, Dunedin, New Zealand, <sup>4</sup>Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand, <sup>5</sup>Eye Department, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand.

Introduction: Both coding loss-of-function mutations of *FOXL2* and remote *cis*-regulatory deletions of the *FOXL2* region lead to the rare, autosomal dominant disorder blepharophimosis syndrome (BPES), associating an eyelid malformation with premature ovarian insufficiency. Despite extensive genetic studies, the molecular cause remains unexplained in 12% of typical BPES patients.

Patients and Methods: The goal of this study was to unravel the molecular cause in a multigenerational Polynesian family in which linkage to *FOXL2* (LOD of 3.8) was shown. *FOXL2* and its entire *cis*-regulatory domain (chr3:138652808-139067278;GRCh37) was enriched using HaloPlex followed by next-generation sequencing in five individuals. Data-analysis was performed using CLCbio.

**Results:** We identified a heterozygous non-coding variant, Chr3(3RCh37):g.138954755G>A, in a non-conserved sequence, predicted to function as an enhancer by Epigenome Roadmap and Ensembl. In addition, this predicted enhancer is located in the shortest region of overlap of previously delineated *cis*-regulatory deletions. An interaction of this fragment with the *FOXL2* promoter has been demonstrated using Chromosome Conformation Capture (3C) in human granulosa-like tumor KGN cells (D'haene et al. PLoS Genetics 2009).

**Conclusions:** This is the first report of a non-coding variant in a putative novel enhancer of *FOXL2* leading to BPES. Our study adds to the increasing number of Mendelian developmental disorders caused by subtle genetic defects of *cis*-regulatory elements, such as the ZRS and SIMO elements in the *SHH* and *PAX6* regions respectively.

This work is supported by the Research Foundation Flanders (FWO). H.V. is a postdoctoral fellow and E.D.B. is a senior clinical investigator of the FWO.

### P13.36

#### Functional analyses of mutations in the mTOR regulator NPRL3 which are responsible for focal epilepsy and cortical dysplasia

**M. Eggert**, D. Buschmann, S. Müller, O. K. Steinlein;  
Institute of Human Genetics, Munich, Germany.

Recently, mutations in the GATOR1 complex subunit gene NPRL3 were described as new genetic cause for focal epilepsy and cortical dysplasia. NPRL3 together with NPRL2 and DEPDC5 constitute the GATOR1 complex, an important inhibitor of the mechanistic target of rapamycin complex 1(mTORC1). Yet, not much is known about the cellular function of these three GATOR1 proteins. As to mTORC1, it is known that this enzyme complex plays a key role in cellular energy homeostasis, autophagy, as well as in cell growth and cell cycle pathways. Activation of mTORC1 leads to activation of two important translation regulatory enzymes, 4-EPB and S6K. So far, no functional study has been conducted to investigate the mechanisms underlying NPRL3 mutations in focal epilepsy. Therefore, we performed in vitro mutagenesis experiments to investigate the impact of NPRL3 mutations published by us and others on the mTORC1 pathway. The ELISA results indicate that the protein interaction between NPRL3 harboring a mutation and DEPDC5 is not affected. Our western blot analyses revealed phosphorylated 4-EPB and S6K, even under amino acid deprivation condition, indicating loss of function of the mTORC1 inhibitor GATOR1 due to mutated NPRL3. These results indicate that mutations in NPRL3 lead to loss of mTORC1 inhibition. It is therefore likely that NPRL3 is essential for a functional GATOR1 complex.

### P13.37

#### Three cases with partial 14q trisomy detected in abnormal karyotype and more precisely identified by SNP array

**V. Becvarova<sup>1</sup>, V. Curtisova<sup>2</sup>, P. Capkova<sup>2</sup>, V. Hurkova<sup>3</sup>, M. Magner<sup>4</sup>, R. Mihalova<sup>4</sup>, J. Horacek<sup>4</sup>, M. Trkova<sup>1</sup>;**

<sup>1</sup>GENNET, Prague, Czech Republic, <sup>2</sup>University Hospital, Olomouc, Czech Republic,

<sup>3</sup>Prediko, Zlin, Czech Republic, <sup>4</sup>University Hospital, Prague, Czech Republic.

**Introduction:** Chromosome 14 is often involved in chromosomal rearrangements. Partial trisomy of the distal segment can come from intrachromosomal or interchromosomal rearrangements in parental karyotypes or de novo formation. Determination of the unidentified extra material is usually performed by array analyses. Until recently, a few 14q duplication cases were reported. Most common clinical features are low birth weight, intellectual disability, hypotonia, microcephaly, dysmorphia, respiration failure, hypertelorism. However, no specific critical genes in this region are known yet. Reporting patients with similar aberrant chromosomes 14 will be helpful in order to characterize typical clinical genotype - phenotype correlation.

**Material and Methods:** Chromosomal aberrations were detected by standard karyotyping. Array analysis was performed by Illumina HumanCytoSNP 12v2.1; GRCh37/hg19. We report 3 patients of different ages (7 years, 4 months, 22 years) and with different range of duplicated chromosomal segments.

**Results:** Patient 1 - boy 7 years old; arr[hg19] 14q32.1  
1q32.33(91,474,831-107,282,437)x3 dn, patient 2 - girl 4 months;  
arr[hg19] 14q31.1q32.33(83,208,737-107,282,437)x3 dn, patient 3 - woman, 22 years old; arr 12p13.33p13.32(1-5,168,849)x1,14q31.3q32.33(86,991,381-107,283,504)x3 - comes from paternal balanced translocation. Phenotypes of all 3 patients were similar.

**Conclusions:** In three detected partial 14q trisomies different number of genes are included: patient 1 - 140 OMIM genes; patient 2 - 153 OMIM genes; patient 3 - 152 OMIM genes (with concurrent partial deletion 12p). All examined patients share the distal 140 OMIM genes region (15.8 Mb).

Common clinical features as a typical craniofacial dysmorphism, respiration failure and intellectual disability are present in all three patients and phenotype-genotype is discussed.

### P13.38

#### Splicing analysis of exonic OCRL mutations causing Lowe syndrome or Dent-2 disease

**L. Suarez-Artiles, E. Ramos-Trujillo, E. Cordoba-Lanus, F. Claverie-Martin;**  
Unidad de Investigacion, Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.

Mutations in the OCRL gene are associated with both Lowe syndrome and Dent-2 disease. Patients with Lowe syndrome present congenital cataracts, mental disabilities and a renal proximal tubulopathy, whereas patients with Dent-2 disease exhibit similar proximal tubule dysfunction but only mild, or no additional clinical defects. It is not yet understood why some OCRL mutations cause the phenotype of Lowe syndrome, while others develop the milder phenotype of Dent-2 disease. Our goal was to gain new insights into the consequences of OCRL exonic mutations on pre-mRNA splicing.

Thirteen missense mutations and one synonymous mutation located mainly in poorly defined exons and potentially affecting splicing regulatory elements or splice sites were selected. Their effects on splicing were studied using bioinformatics tools and a minigene assay. Specific mutations were introduced by site-directed mutagenesis, and the RNA was analysed by RT-PCR and DNA sequencing.

We found that three presumed missense mutations caused alterations in pre-mRNA splicing. Mutation p.W247C generated a splicing silencer and disrupted a splicing enhancer resulting in skipping of exon 9, while mutations p.A861T and p.A861P abolished a donor splice site and resulted in skipping of exon 23.

In conclusion, these results highlight the importance to evaluate the effects of missense mutations at the mRNA level in Lowe syndrome. Our findings also allowed the detection of previously unpredicted splicing regulatory elements in OCRL exon 9.

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### P13.39

#### Propranolol exerts its effects through cell cycle and proliferation genes on cancer cells

**F. I. Sahin<sup>1</sup>, O. D. Iseri<sup>2</sup>, Y. K. Terzi<sup>1</sup>, F. Sarialioglu<sup>3</sup>;**

<sup>1</sup>Baskent University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey, <sup>2</sup>Baskent University Institute of Transplantation and Gene Sciences, Ankara, Turkey, <sup>3</sup>Baskent University Faculty of Medicine Department of Pediatric Oncology, Ankara, Turkey.

**Introduction:** Utilization of beta adrenergic receptor antagonists in several cancer types have been reported in the recent years. Mechanisms underlying drug effects are being investigated. In a previous study, we demonstrated that propranolol, atenolol and ICI118,551 inhibited cell growth and migration in vitro in three different cell lines. In the current study, we aimed to investigate the effects of propranolol on metastatic breast carcinoma (MCF7) and colon carcinoma (HT29) cell lines.

**Materials and Methods:** We detected beta adrenergic receptor expression in the two cell lines and then analyzed RNA expression levels of 86 different genes playing role in cell cycle and proliferation as well as apoptotic pathways.

**Results:** We noticed that, exposure of both cell lines to propranolol for 24-72 hours resulted in cell death regardless of beta adrenergic receptor expression levels.

**Conclusions:** Thus, for the first time in the literature, we concluded that propranolol exerts its effect by inhibiting or activating the expression of multiple genes involved in cell proliferation and death.

This study has been approved by Baskent University Institutional Review Board (Project No: DA15/09) and supported by Baskent University Research Fund.

**P13.40****Altered pre-mRNA splicing due to missense CLDN16 and CLDN19 mutations associated with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis**

*A. Perdomo-Ramirez, M. de Armas-Ortiz, E. Ramos-Trujillo, H. Gonzalez-Acosta, F. Claverie-Martin;  
Unidad de Investigación, Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.*

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal-recessive renal tubular disorder characterized by excessive urinary losses of magnesium and calcium, bilateral nephrocalcinosis and progressive chronic renal failure. This rare disease is caused by mutations in CLDN16 or CLDN19. Patients with mutations in CLDN19 also present severe ocular abnormalities. Most of these mutations are predicted as missense. However, it is known that a large fraction of exonic mutations can alter pre-mRNA splicing. In this study, we tested presumed missense mutations in these genes for their effects on splicing.

Bioinformatics tools were used to select mutations with potential effect on splicing. Variants were experimentally tested using minigene assays. Specific mutations were generated by site-directed mutagenesis. RNA from cultured cells was analyzed by RT-PCR and automatic DNA sequencing.

Seven CLDN16 mutations; p.L145P, p.R149Q, p.R149L, p.L151F, p.L151W, p.G198D and p.G198A and two CLDN19 mutations; p.G130C and p.G130D were analyzed. RT-PCR results showed that p.G198A, p.G198D and p.G130C produced skipping of exons 4 and 2, respectively. We also found that mutations p.R149L and p.L151F resulted in loss of an exon 3 fragment.

Our results indicate that some presumed missense mutations act as splicing mutations. These nucleotide substitutions represent the first exonic mutations that induce aberrant mRNAs in FHHNC. These findings strengthen the significance to evaluate the consequences of missense mutations at the RNA level.

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**P13.42****Whole genome sequencing of spermatocytic tumour (SpT), a rare testicular tumour at the crossroads between somatic and germline mutational processes**

*G. J. Maher<sup>1</sup>, E. Giannoulatou<sup>1,2</sup>, Z. Ding<sup>1</sup>, E. Rajpert-De Meyts<sup>3</sup>, A. J. M. Gillis<sup>4</sup>, L. C. J. Dorssers<sup>4</sup>, A. WGS500<sup>5</sup>, G. McVean<sup>6</sup>, L. H. Looijenga<sup>4</sup>, A. O. M. Wilkies<sup>1</sup>, A. Gorleby<sup>1</sup>;*

<sup>1</sup>Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom, <sup>2</sup>Victor Chang Cardiac Research Institute, Darlinghurst, Australia,

<sup>3</sup>Department of Growth & Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, <sup>4</sup>Department of Pathology, Erasmus MC - University Medical Center Rotterdam, Rotterdam, Netherlands, <sup>5</sup>University of Oxford, Oxford, United Kingdom, <sup>6</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Spermatocytic tumour (SpT) is a rare testicular tumour that is clinically distinct from the common classical seminoma. With a median age of onset ~55y, it is the only germ cell tumour of postnatal origin and thus represents a useful model to study human germ cell biology and the characteristics of germline mutations. We performed whole genome sequencing on 4 SpTs (52x coverage) and matched normal tissue (26x). The tumours exhibit extensive aneuploidy (50-99 autosomes/tumour) unusually involving whole chromosomes, with relative gains of chr9 and chr20 common to all tumours. Genome-wide, the acquired single nucleotide variant (SNV) load was extremely low for these adult-onset tumours (~0.2 per Mb). An average of 6 (2-9) non-synonymous variants were called per tumour; no SNVs were shared across tumours and no known oncogenic drivers were identified. Strikingly, in all cases, mutant allelic ratios were low (<50%) and inversely correlated with chromosomal copy-number, suggesting that they represent secondary passenger mutations. Two-thirds of SNVs were transitions, with C>T accounting for 51% of all SNVs, most commonly occurring at CpGs; a bias to specific trinucleotide contexts was observed. This mutational signature, which is distinct from other germ cell tumours, is typical of that observed for de novo germline mutations. We propose that in this unique tissue, gene imbalance caused by chromosomal aneuploidy is the oncogenic mechanism driving tumourigenesis and results in spermatogonia's re-entry into mitosis following failure to complete meiotic divisions. This work is supported by the Wellcome Trust 091182 (to AG and AOMW) and 102731 (to AOMW).

**P13.43****Analyses of splicing mutations in the SFTPC gene: example of the p.Gln145His mutation**

*C. Delestrain<sup>1,2</sup>, S. Simon<sup>1</sup>, A. Aissat<sup>1,3</sup>, R. Medina<sup>3</sup>, X. Decrouy<sup>1</sup>, B. Costes<sup>1</sup>, R. Epaud<sup>1,2</sup>, P. Fanen<sup>1,3</sup>;*

<sup>1</sup>INSERM U955, Creteil, France, <sup>2</sup>Centre Hospitalier Intercommunal de Creteil, Service de Pédiatrie Générale, Creteil, France, <sup>3</sup>AP-HP, Hôpital Henri Mondor, Département de Génétique, Creteil, France.

Since the first description of interstitial lung disease (ILD) being associated with an alteration of the SFTPC gene in 2001, about 50 different mutations have been reported. All patients are heterozygous carriers of autosomal dominant SFTPC mutations and the lung disease caused by different SFTPC mutations covers a broad range of phenotypes from neonatal respiratory distress syndrome to adult ILD. The wide phenotypic variability is not fully explained by the genotype and we postulated that splicing defect could explain such a discrepancy.

We first study an exonic mutation, c.435 G> C (p.Gln145His) localized in the well conserved BRICHOS domain of the pro-protein SP-C (proSP-C) and associated to a fatal neonatal respiratory distress syndrome. In silico analysis predicted a drastic reduction of the splice donor site at the exon-intron 4 junction's strength score. Using wild-type and mutant minigenes transfected in alveolar type II epithelial cells (A549), we showed a complete exon skipping due to this mutation. When co-transfection of both wildtype and mutant minigenes was performed, a dominant negative effect on the splicing was observed. Exon 4 is in frame and its skipping lead to a truncated protein which is retained in the endoplasmic reticulum as shown by confocal microscopy.

These results indicated that the mutation p.Gln145His (Q145H), previously described as a missense mutation, is actually a splicing mutation which should be referred as c.325\_435del (p.Leu109\_Gln145del). The absence of alternative splicing leading to the skipping of exon 4 fully explained the severity of the phenotype observed.

**P13.44****A comparative analysis of functionality between a recombinant thermostable reverse transcriptase and a commercial mesophilic reverse transcriptase using RT-PCR and Real time PCR**

*N. Faramarzi Garous, S. Gharavi, Z. Moosavi Nejad, N. Ghorbanmehr, M. Sheikh Hosseini;*

*Alzahra University, Tehran, Iran, Islamic Republic of.*

Reverse Transcriptase, is an important enzyme involved in the synthesis of cDNA from unstable RNA molecules and has applications in transcriptome and RNA expression profiles. The efficiency of this enzyme relies on its molecular characteristics, including those that confer thermostability, processivity and error-free reverse transcription capabilities. cDNA production at high temperatures has several advantages like elimination of secondary structures in the RNA molecule and use of RNA with GC-rich regions in templates.

A bacterial intronic gene coding for thermostable reverse transcriptase (*trt*) has been identified and cloned in pET28a vector and subsequently expressed in a suitable host (*E.coli* BL21). Following induction of the bacterial culture and sonication of the cells, the enzyme was purified by Ni+2 columns.

The efficacy of the recombinant enzyme was successfully tested in RT and Nested-PCR reactions at 37, 60, 70, 80 and 90°C for the detection of *trt* 9:22. The capabilities of this enzyme was also compared to the mesophilic M-MLV RT and proved to be more versatile and robust. Specific activity in optimum conditions compared for *Trt* and MMLV enzymes by Real time-PCR. The optimum temperature for *Trt* activity was 70 °C. A significant increase compared to optimal temperature of MMLV activity (37 °C) is shown.

The *Trt* protein can carry out reverse transcription at very hot temperatures (cDNA synthesis was detectable at 90°C) and is a novel type of RT that may be exploited for applications where synthesis of cDNA at high temperatures is preferable to diagnose CML, ALL, AML diseases.

**P13.45****De novo translocation frequency of the recurrent constitutional t(11;22)(q23.1;q11.21) in normal somatic tissues**

*H. Kurahashi, T. Kato, M. Suzuki, M. Tsutsumi, T. Ohye, H. Inagaki;*

*Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan.*

The t(11;22)(q23.3;q11.2) is one of several recurrent constitutional translocations mediated by palindromic AT-rich repeats (PATRRs). Although it is likely that the secondary structure triggers the initiation of the translocati-

on formation, precise underlying mechanism remains enigmatic. In this study, we carried out the t(11;22)-specific PCR for the der(11) or the der(22) using primers flanking the PATRR11 and PATRR22. To analyze the de novo t(11;22) in the genomic DNA from normal individuals, the PCR was carried out to detect the t(11;22) at the single cell sensitivity. No translocation was detected in the DNA from somatic tissues including stomach, small intestine, colon, spleen and bone marrow. When we use DNAs from testis or ovary from fetus and adult, only the testicular tissue from adult showed the t(11;22)-specific PCR products at a lower frequency than that generally observed in normal semen. DNA from eight cancer or leukemia cell lines were also negative for the PCR. Our data suggest the hypothesis that non-replicative mechanism for formation of the recurrent palindrome-mediated translocations.

#### P13.46

#### Unbalanced de novo X;13 translocation with skewed X chromosome inactivation

M. Orera<sup>1</sup>, C. Rodriguez Hernandez<sup>1</sup>, L. Fernandez Garcia Moya<sup>2</sup>, M. Mori<sup>2</sup>, V. Seidel<sup>1</sup>, <sup>1</sup>Hospital Gregorio Marañón, Madrid, Spain, <sup>2</sup>Hospital La Paz, Madrid, Spain.

**Introduction:** X chromosome inactivation is meant to provide an equal gene dosage between males and females. X-autosome translocations are rare events. When the translocation yields an unbalanced genomic rearrangement, the derivative X chromosome is generally selectively inactivated, avoiding the effect of the autosomal trisomy. We present a girl with an unbalanced X-13 translocation.

**Materials and Methods:** 4th child of non-consanguineous parents. The mother was treated with thyroid hormone and hydroxychloroquine during the pregnancy. A C- section was performed for fetal distress at 38 weeks, with birth weight 2580 g, length 49 cm. She presents postaxial polysyndactyly on the left foot, anteriorly placed anus and a sacral dimple. Abdominal USS detected a bicornuate uterus. At age 12 months a mild developmental delay was noted. Her overall growth remains between 3rd and 10th centiles.

**Results:** Standard karyotype: 46,X,der(X)t(X;13)(p11;q12.1) Chromosome microarray: 13q12.3-q34 duplication (84 Mb) and Xp22.33-Xp11.23 deletion (46 Mb)

X inactivation studies showed a markedly skewed inactivation pattern.

Parental karyotypes were normal.

**Conclusions:** The mild clinical phenotype of partial trisomy 13 together with the skewed X inactivation pattern in blood suggests that the derivative X chromosome carrying a large segment of chromosome 13 is preferentially inactivated in most tissues. Mild features of partial Xp monosomy are to be expected as the deletion affects PAR.

To our knowledge, this is the first report of a child with almost complete trisomy 13 originated by a de novo X;13 translocation.

#### P13.47

#### Identification of chromosomal translocation breakpoints associated with holoprosencephaly using whole-genome sequencing: Suggestion of a positional effect.

J. Klar<sup>1,2</sup>, H. Ehrencrona<sup>3</sup>, N. Dahl<sup>1,2</sup>;

<sup>1</sup>Department of Immunology, Genetics and Pathology, Uppsala, Sweden, <sup>2</sup>Science for Life Laboratory, Uppsala, Sweden, <sup>3</sup>Division of Clinical Genetics, Lund, Sweden.

**Introduction:** We identified a boy with developmental delay, diplegia and lobar holoprosencephaly (HPE). Karyotyping revealed an apparently balanced reciprocal *de novo* chromosomal translocation t(10;12)(q24;p13). Genome-wide array analysis (Affymetrix CytoScan HD) and exome sequencing did not detect any pathogenic variants.

**Materials and Methods:** We performed whole-genome sequencing (WGS) using Illumina TruSeq (insert size of 350 bp) on a HiSeqX (paired-end and 150bp read length). To identify breakpoints we utilized BreakDancerMax, a Perl/C++ package that provides genome-wide detection of structural variants. Anomalously aligned reads at chromosome 10q24 and chromosome 12p13 were visualized using Integrative Genomics Viewer (IGV) and confirmed by Sanger sequencing.

**Results:** At the breakpoint on the chromosome 10 derivative, we identified a deletion of 7 bp and an insertion of 20 bp of unknown origin. Likewise, at the breakpoint on the chromosome 12 derivative, we identified a deletion of 31 bp followed by an insertion of 14 bp of unknown origin. Both breakpoints are situated in intergenic regions, specifically between the genes *FBXW4* and *FGF8* on chromosome 10q24 and between *EFCAB4B* and *PARP11* on chromosome 12p13. According to Decipher database, insertion and deletions spanning both breakpoint regions are associated with intellectual disability and macro/microcephaly. Furthermore, variants in *FGF8* have been reported in autosomal recessive HPE.

**Conclusions:** Traditional methods to determine translocation breakpoints, such as FISH, Southern blot or long-range PCR, are laborious and with limited resolution. Our results demonstrate the efficacy of WGS for the precise identification of translocation breakpoints in non-coding regions.

#### P13.48

#### When genotype does not account for phenotype: the lesson from isodisomic chromosomes

A. La Barbera<sup>1</sup>, A. Provenzano<sup>2</sup>, M. Garonzi<sup>3</sup>, E. Zago<sup>4</sup>, V. Orlandini<sup>2</sup>, M. Pantaleo<sup>1</sup>, G. Traficante<sup>2</sup>, M. Vignoli<sup>5</sup>, G. Bacci<sup>6</sup>, S. Guarducci<sup>1</sup>, S. Bargiacchi<sup>2</sup>, E. Gambineri<sup>5</sup>, M. Delledonne<sup>3,4</sup>, O. Zuffardi<sup>7</sup>, S. Giglio<sup>1,2</sup>;

<sup>1</sup>Medical Genetics Unit, Meyer Children's University Hospital, Florence, Italy, <sup>2</sup>Medical Genetics Unit, Department of Clinical and Experimental Biomedical Sciences, Mario Serio, University of Florence, Florence, Italy, <sup>3</sup>Department of Biotechnology, University of Verona, Verona, Italy, <sup>4</sup>Personal Genomics srl, Verona, Italy, <sup>5</sup>Department of „NEUROFARBA,“ Section of Children's Health, University of Florence, Anna Meyer Children's Hospital, Florence, Italy, <sup>6</sup>Ophthalmology Unit, Department of Neuroscience, Meyer Children's Hospital, Florence, Italy, <sup>7</sup>Department of Molecular Medicine, University of Pavia, Pavia, Italy.

We demonstrated isodisomy for chromosome 1 in two unrelated subjects from non-consanguineous parents, affected by autosomal recessive syndromes associated with two genes on chromosome 1, type 2 nephrotic syndrome (#600995) and campodactyly-arthropathy-coxa vara-pericarditis (#208250). We detected an homozygous mutation in NPHS2 (1q25.2,c.538G>A) in case 1, and in PRG4 (1q31.1,c.2288C>G) in case 2, inherited only by the father. SNP-CGH array analysis showed complete homozygosity for chromosomes 1 in both cases. This finding is in agreement with post-zygotic monosity rescue of the paternal chromosome 1, as a consequence of maternal non-disjunction of chromosomes 1. To exclude recessive conditions for other genes located on chromosome 1, we performed whole exome sequencing. We identified variants in FUCA1, DPYD, ABCA4, NPHP4, PINK1 (case 1) and DDOST, STIL, ATP7B, CFH genes (case 2), considered disease-causative by HGMD and never reported in dbSNP and Exac. A detailed clinical examination in both patients did not show any signs of the expected diseases, excepted for PINK1 that is associated with a late onset disease. Our observations further demonstrate that low penetrance and extensive phenotype heterogeneity occur even for genomic variants reported as causative in homozygous state. The analysis of entire exome/genome is a major challenge to geneticists in order to provide genotype-phenotype correlations, especially in prenatal setting.

#### P13.49

#### Transcriptome Sequencing and characterization of two Bloom's syndrome patients

M. M. Montenegro, G. M. Novo-Filho, A. M. Nascimento, E. A. Zanardo, A. T. Dias, T. V. M. M. Moura, C. Milani, F. Madia, D. A. M. Silvestre, C. A. Kim, L. D. Kulikowiski; FMUSP, São Paulo, Brazil.

**Introduction:** Transcriptome profiling of mRNA could be a powerful approach to identify new transcripts, fusion genes, gene regulation, mutations and networks of genes that play a role in diseases. We investigated relevant genomic markers that could be associated to Bloom's syndrome (BS), a chromosomal instability disorder caused by mutations in BLM gene. **Materials and Methods:** We performed a deep-sequencing RNA-Seq profiling using high throughput sequencing (Illumina HiSeq 2500 platform) of samples derived from two patients with BS and three unaffected controls. The raw data analysis was generated using specialized softwares (CASAVER 1.8.2, Bowtie2, EdgeR, Rsubread and DESeq2). **Results:** The RNA-Seq assay revealed the precise location of transcription limits, with resolution of a single nucleotide and high level of efficiency, showing high genetic complexity. The results disclosed 399 genes differentially expressed: 216 up regulated in the group with Bloom syndrome and 183 up regulated in the control group. Unexpectedly most of them were immune system-related genes. Also, we detected large number of SNP and IDELS in one of BS patients, highlighting a frameshift in the BLM gene. **Conclusions:** Our results suggested that gene expression network in BS could interfere in the regulation of the pathways associated with the immunological systems regulation probably caused by disturbance of DNA repair mechanisms. Furthermore, the study of the transcriptome using RNA-Seq may help to a breakthrough in the pathogenesis of BS. Grants: FAPESP: 09/53105-9 and FINEP-CT INFRA 0160/12 SP8.

## P14 New diagnostic approaches, technical aspects & quality control

### P14.002

#### C26-ceramide is a new and sensitive biomarker for Farber's disease

C. Cozma<sup>1</sup>, M. I. Iurascu<sup>1</sup>, S. Eichler<sup>1</sup>, S. Zielke<sup>2</sup>, A. Rolfs<sup>2</sup>, J. Köster<sup>1</sup>;

<sup>1</sup>Centogene AG, Rostock, Germany, <sup>2</sup>Albrecht-Kossel-Institute, University of Rostock, Rostock, Germany.

Farber disease (Farber's lipogranulomatosis, ceramidase deficiency), is an autosomal recessive, extremely rare disease caused and characterized by a deficient acid ceramidase activity encoded by ASAHI gene. Low ceramidase activity is resulting in accumulation of fatty substances, mainly ceramides. At clinical level, Farber disease is manifesting through hallmark symptoms such as: periarticular nodules, lipogranulomas, swollen and painful joints and a hoarse voice or a weak cry; in addition to these, also hepatosplenomegaly, rapid neurological deterioration or developmental delay are reported [1, 5-6]. Seven different Farber types were described, with phenotypes varying from mild cases with a longer life expectancy to very severe cases, where the patients do not survive past their first year of life.

The screening through over 40 different ceramide-like molecule show that only C26 is specifically increased in samples from Farber patients. We present here a new method of diagnosis of Farber disease by determining the concentration of C26 ceramide isoforms using LC/MRM-MS and C25 ceramide as internal standard. Moreover, we found that cis-isomer of the C26 ceramide is a specific biomarker for Farber disease, with pathological values in a range of 39.2-150.0 nmol/L blood (normal range 13.6-23.4 nmol/L blood, N=192, healthy individuals). The new biomarker can be determined directly in the dried blood spot extract with low sample consumption, easy sample preparation, high reproducibility and it presents the possibility of to be used in high throughput screenings.

### P14.004

#### Development of a new biomarker method for the diagnosis of MLD using tandem mass spectrometry

S. Eichler<sup>1</sup>, C. Cozma<sup>1</sup>, M. I. Iurascu<sup>1</sup>, A. Rolfs<sup>2</sup>;

<sup>1</sup>Centogene AG, Rostock, Germany, <sup>2</sup>Albrecht-Kossel-Institute, University of Rostock, Rostock, Germany.

Metachromatic leukodystrophy (MLD) is a lysosomal storage disease with an autosomal recessive inheritance pattern. MLD is caused by a deficiency of the enzyme arylsulphatase A (ARSA) and is characterized by enzyme activity in leukocytes that is less than 10% of normal controls. However, assay of the ARSA enzyme activity alone is not sufficient for diagnosis; ARSA pseudodeficiency, which is characterized by enzyme activity that is 5~20% of normal controls does not cause MLD. Without this enzyme, sulfatides build up in many tissues of the body, eventually destroying the myelin sheath of the nervous system with serious consequences manifested in clinical symptoms e.g. weakness, muscle rigidity, developmental delays, convulsions, paralysis, and dementia. A recent study contended sulfatide is not completely responsible for MLD because it is nontoxic. It has been suggested lysosulfatide plays a role because of its cytotoxic properties in vitro. Recently developed enzyme replacement therapy and the steep progression of the disease in ARSA patients make the development of HTS diagnosis of high importance.

Here we report the development of a new biochemical screening method for ARSA patients using a combination of two techniques: (i.) arylsulphatase enzymatic activity and (ii.) quantification of ARSA specific biomarkers (both lyso-sulfatide and selected sulfatides). Both assays are using for the analysis liquid chromatography coupled with high resolution multiple reaction monitoring mass spectrometry (LC/MRM-MS) and the results were confirmed by genetic analysis. This screening method has been validated for dried blood spot (DBS) technology which further simplifies the sample handling, processing and analysis.

### P14.005

#### The use of automated facial analysis in the clinical diagnostic of autism spectrum disorders (ASD)

N. A. Varga, P. Balicza, K. Pentelenyi, H. Piko, M. Szegedi, V. Karcagi, M. Molnar; Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary.

Introduction: Autism can be part of a genetic syndrome associating with dysmorphic features. While there are many studies that described genetic alterations associated with ASD, none have identified facial phenotypes associating to ASD with computer-aided facial analysis. Here we evaluate the geno/phenotype relation in a limited number of autistic individuals.

Methods: An image set of affected individuals divided into two cohorts: presenting facial dysmorphologies (AFD) (n=20) and without dysmorphology (ANFD) by human experts. These cohorts were compared to unaffected controls (n=50) using variables and rankings produced by the Facial Dysmorphology Novel Analysis (FDNA) technology. In both ASD cohorts mutations of 103 genes associated to autism, repeat expansions of FMR1 gene, and in some AFD cases chromosomal abnormalities have been identified.

Results: Clear trends were recognizable in the three groups along facial dysmorphology variables and rankings. Although labeled as not showing facial dysmorphology, the ANFD cohort was automatically ranked separately from the unaffected controls. In addition, two possible clusters of ANFD were recognized by the technology. The AFD cohort was clearly recognized as separate from the other two cohorts. In the AFD group Fragile X, CHARGE, Silver Russell, Cohen, 2q37.3 microdeletion syndrome, and Speech Language Disorder 1 were identified.

Conclusion: The preliminary results show that deep phenotyping provided by a computer-aided facial analysis could be instrumental in the early clinical diagnostic of the syndromic ASD forms and could help in the selection of diagnostic molecular tests. Larger study is needed to assess these trends and to allow cross-validation.

### P14.006

#### A comparison of two NGS novel targeted panel-sequencing assays for patients with autoinflammatory disease

M. Antolín<sup>1</sup>, H. Valbuena<sup>1</sup>, A. Fernández Rodríguez<sup>1</sup>, A. Outeiral<sup>1</sup>, A. M. Cordero<sup>1</sup>, E. García-Arumí<sup>1,2</sup>, E. Tizzano<sup>1,2</sup>;

<sup>1</sup>Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron, Barcelona, Spain, <sup>2</sup>Biomedical Network Research Centre on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.

The molecular diagnosis of autoinflammatory diseases includes several candidate genes such as *MEFV*, *MVK*, *TNFRSF1A*, *NOD2* and *NLPR3*. NGS offers an improvement in time and costs versus Sanger sequencing. We compared two different NGS approaches for molecular diagnosis of these patients.

Two different library approaches were followed. In the first, 8 different fragments *MEFV* (14.600 kb), *MVK* (23.576 kb), *TNFRSF1A* (13.358 kb), *NOD2* (35.939 kb) and *NLPR3* (31.054 kb) were amplified using Takara *LA Taq* DNA Polymerase and library preparation was performed using NexteraXT. In the second, a Custom GeneRead DNAseq panel of targeted genes was amplified (197 amplicons with a mean size of 203 bp) and libraries were prepared with NEBNext Ultra DNA kit. In both approaches, libraries were pooled and sequenced using paired-end on the MiSeq platform. Sequencing data were analyzed with the Variant Studio and GeneRead Seq Variant Analysis software, respectively. The validation of the potential pathogenic variants was performed by Sanger sequencing.

The Nextera approach requires a high quality of DNA to amplify fragments until 15000 bp. The sequences include the whole intronic regions, but the coverage is very heterogeneous. On the other hand, GeneRead protocol is fast, it predicts the uncovered regions and the coverage is similar and constant between runs. In both procedures, the detected variants were highly similar and false positives have not been observed.

Both approaches are useful in the detection of variants associated with the pathogenesis of autoinflammatory diseases. In our hands, Generead appears faster and more reproducible between runs.

### P14.007

#### A novel mutation in *WDR62* gene identified in a Moroccan family with autosomal recessive primary microcephaly using Next - Generation Sequencing

I. Cherkaoui Jaouad<sup>1,2</sup>, A. Zrhidri<sup>1</sup>, W. Jdioui<sup>1,2</sup>, J. Lyahyai<sup>1</sup>, L. Raymond<sup>3</sup>, M. Taoudi<sup>3</sup>, G. Egea<sup>3</sup>, S. El Mouatassim<sup>3</sup>, A. Sefiani<sup>1,4</sup>;

<sup>1</sup>Centre de Génomique Humaine, Faculté de médecine et de Pharmacie, Rabat, Morocco,

<sup>2</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco,

<sup>3</sup>Département de Génétique Moléculaire, Laboratoire Biomnis, Lyon, France, <sup>4</sup>Institut National d'Hygiène, Rabat, Morocco.

Introduction: Autosomal recessive primary microcephaly (MCPH) is a rare genetically heterogeneous disorder of neurogenic brain development characterized by reduced head circumference at birth with no gross anomalies of brain architecture and variable degrees of intellectual impairment. Clinical and genetic heterogeneity in monogenetic disorders represents a major diagnostic challenge.

Here, we used the whole-exome sequencing as a diagnostic approach for establishing a molecular diagnosis in a family with two children with MCPH. Materials and Methods: Two patients, 11 and 9 years old, born from consanguineous parents, were referred to the department of medical genetics. The diagnosis of MCPH was made, based on reduced head circumference without brain architecture abnormalities. Whole-exome sequencing was per-

formed in these patients, their parents and their two healthy sibling.

Results: A homozygous mutation c.1027C>T; p.Gln343Ter in exon 8 of WDR62, a gene already known to be related to MCPH, was identified. Sanger sequencing confirmed this mutation in the affected children. This mutation was not found in HGMD and 1,000 Genome database.

Conclusions: Our data expanded the spectrum of mutations in WDR62 gene, and give more arguments of the powerful and cost- effective tool of whole exome sequencing for the molecular diagnosis of genetically heterogeneous disorders such MCPH. Exome sequencing allows us to have faster molecular results especially when specific diagnosis needs a longer time to sequence many genes.

#### P14.008

#### Development of a next generation sequencing panel for diagnostic and investigation of bleeding disorders

*N. Borràs<sup>1,2</sup>, L. Ramírez<sup>1,2</sup>, N. Comes<sup>1,2</sup>, C. Altisent<sup>3</sup>, R. Parra<sup>1,3</sup>, F. Vidal<sup>1,2</sup>, I. Corrales<sup>1,2</sup>;*

<sup>1</sup>Blood and Tissue Bank, Barcelona, Spain, <sup>2</sup>Vall d'Hebron Research Institute, Barcelona, Spain, <sup>3</sup>Vall d'Hebron University Hospital, Barcelona, Spain.

The advent of NGS has opened the possibility to design custom protocols for molecular analysis of several genes in parallel. This approach is particularly valuable in the diagnosis of diseases with similar phenotype although caused by a heterogeneous molecular basis. Taking advantage of this novel opportunity, we designed and validated a NGS custom panel to simultaneously analyze the 23 essential genes involved in inherited bleeding disorders.

The targeted exon enrichment GeneRead panel (QIAGEN) designed comprises a total of 1,285 amplicons (size average 169 bp) covering the 98.7% of the target genomic regions. Construction of libraries, including patient-specific indexation, was performed with NEBNext Ultra DNA Library Prep Kit. Between 24 and 48 libraries were sequenced together in every MiSeq (Illumina) run. To date, we have been diagnosed in this way more than 250 patients, suffering both frequent and minor coagulopathies. Putative mutations were identified by GeneRead Variant Calling software and further validated by Sanger methodology, reaching 100% sensitivity. This versatile protocol allows to process together samples from different pathologies, with the consequent simplification of routine procedure and cost reduction. Likewise, it has been especially advantageous when clinical diagnosis was unclear or controversial due to a borderline phenotype with various candidate genes. Importantly, this methodology offers invaluable genetic information of all the variants, polymorphisms and causative mutations, from several hemostatic genes. Analysis and integration of this data could refine the knowledge of genotype-phenotype correlation and improve the forecasting of hemorrhagic risk.

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#### P14.009

#### Novel thermal cycler amplifies the entire BRCA1 gene within 10 minutes

*R. Cammaert<sup>1</sup>, M. Kribbe<sup>2</sup>, W. H. Deelen<sup>2</sup>, R. Hofstra<sup>2</sup>, G. de Vos<sup>1</sup>;*

<sup>1</sup>Molecular Biology Systems, Goes, Netherlands, <sup>2</sup>Erasmus Medical Center, Rotterdam, Netherlands.

Background: The most commonly used technique in clinical genetics is PCR. PCR is reasonably time consuming. Most labs perform maximal 4 PCR's per day (including one overnight). Making PCR faster has been accomplished by introducing faster Peltier based equipment and using faster chemistry. Here we report a novel thermal cycler, which can amplify the complete BRCA1 gene under 10 minutes, reducing the amplification time from 150 minutes to less than 10 minutes.

Methods: Amplification is performed in ultra thin wells (<50 micron) in a 96-well microplate format. The PCR machine consists of three temperature zones for denaturation, annealing and extension. Each zone consists of two opposite heated blocks. When the tray enters a temperature zone, the heated blocks are brought together, thus squeezing the wells. The reaction mix reaches the desired temperature instantaneously.

Results: 29 fragments, covering all the exons of the BRCA1-gene were amplified in under 10 minutes, employing 30 cycles over 3 temperatures. Results were confirmed by Sanger sequencing. Moreover, a 102 basepair fragment was amplified in less than 2 minutes, employing 30 cycles over 3 temperatures.

Discussion and conclusion: This technology introduces a microplate compatible PCR format, which can perform most PCR's in less than 10 minutes. It will allow laboratories to change their logistics (a machine is always available shortly), increase their throughput or speed up their process. With this technology one machine comfortably performs over 40 PCR's in an 8-hour working day.

#### P14.010

#### Clinical validity, reproducibility, and utility of NGS panel tests for hereditary breast and ovarian cancer: challenges and solutions

*S. Lincoln<sup>1</sup>, A. Desmond<sup>2</sup>, A. Kurian<sup>3</sup>, D. Haussler<sup>4</sup>, S. Yang<sup>1</sup>, M. Cline<sup>4</sup>, S. Aradhya<sup>4</sup>, J. Ford<sup>3</sup>, L. Ellisen<sup>2</sup>, B. Paten<sup>4</sup>, R. Nussbaum<sup>1</sup>;*

<sup>1</sup>Invitae, San Francisco, CA, United States, <sup>2</sup>Massachusetts General Hospital, Boston, MA, United States, <sup>3</sup>Stanford University Medical Center, Palo Alto, CA, United States,

<sup>4</sup>University of California, Santa Cruz, CA, United States.

Background: In studies of over 1000 patients [1,2] we recently demonstrated that multigene testing can produce clinically valid results, comparable to traditional tests while expanding patient management impact. Other findings included (a) that pathogenicity classifications produced using publicly available resources under recent guidelines [3] were 99.8% similar to those produced by others using a large proprietary database. Also (b) many pathogenic variants were technically challenging for NGS. Here, we further explore these topics in a substantially larger data set.

Methods: De-identified panel test results for roughly 20,000 individuals were examined. In addition, data from the ClinVar database, representing another approximately 20,000 patients, were used.

Results: Consistent with our prior results, about 4-5% of patients harbor mutations in genes other than BRCA1/2. Most of these findings are consistent with the patient's personal/family history and are not incidental. About 10% of these mutations are of types known to be challenging for NGS (e.g. single exon CNVs, large indels, complex events, etc.). These require specialised algorithms or NGS biochemistries to accurately report.

Considering BRCA1/2 variants, interpretations are highly concordant (98.5%) between established diagnostic labs in terms of clinical management impact. Moreover all of the discordances are in rare variants that appear in few patients. Thus, 99.8% of patients are expected to be concordant, as we observed previously.

Conclusions: When using appropriate NGS and variant interpretation methods, validity and utility of panels tests can be established.

1. Desmond, JAMA Oncology 2015

2. Lincoln, J Mol Diag 2015

3. Richards, Genet Med 2015

#### P14.011

#### Cell-free DNA as a tool for non-invasive cancer detection

*A. Montaner<sup>1,2</sup>, J. González<sup>2</sup>, L. Armengol<sup>1</sup>, J. Rodríguez<sup>1</sup>;*

<sup>1</sup>qGenomics Laboratories, Esplugues de Llobregat, Spain, <sup>2</sup>Bioinformatic Research Group in Epidemiology, Centre de Recerca en Epidemiologia Ambiental (CREAL), Barcelona, Spain.

Introduction: Early detection is key for increasing survival in cancer patients but it is limited by the sensitivity of current detection methods. In this context, cell-free DNA (cfDNA), currently used as a non-invasive tool for aneuploidy detection in prenatal screening and graft rejection monitoring, promises to improve early cancer detection. Because cfDNA levels in blood are very low, sample preparation must be optimized. Here we set up a workflow for generating high quality libraries from low cfDNA inputs that can be sequenced using next generation sequencing (NGS) platforms.

Materials and methods: Blood samples from healthy individuals, pregnant women and cancer patients were collected in Streck tubes to avoid genomic DNA contamination from white blood cells. Plasma was separated by centrifugation prior to cfDNA extraction. Multiple cfDNA extraction methods were tested. Library construction was optimized for low cfDNA inputs and samples were analyzed by qPCR and targeted NGS.

Results: Bead and column-based methods produced similar yields (2.5 - 10 ng/ml) but the bead-based methods were preferred for being faster and not requiring the addition of carrier molecules. Using this workflow we were able to detect fetal cfDNA by qPCR, and to successfully sequence libraries constructed from 1ng input DNA.

Conclusions: We have set up a workflow that allows the analysis of limiting amounts of cfDNA through NGS, which can be applied to the detection of biomarkers present in cfDNA in samples from multiple biological origins, including cancer.

#### P14.012

#### Molecular genetic testing and the future of Clinical Genomics

*A. Nuzzo<sup>1</sup>, D. Katic<sup>1</sup>, M. Dulovits<sup>1</sup>, G. Rosenauer<sup>1</sup>, P. Miglinci<sup>1</sup>, M. Uendemir<sup>1</sup>, S. Pabinger<sup>2</sup>, E. Heitzer<sup>3</sup>, C. Bock<sup>4</sup>, G. Hoermann<sup>5</sup>, M. Speicher<sup>6</sup>, A. Kriegner<sup>1</sup>;*

<sup>1</sup>Platomics GmbH, Vienna, Austria, <sup>2</sup>Health and Environment Department, Molecular Diagnostics, AIT Austrian Institute of Technology, Vienna, Austria, <sup>3</sup>Institute of Human Genetics, Medical University of Graz, Vienna, Austria, <sup>4</sup>CeMM Research Centre for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, <sup>5</sup>Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

**Introduction:** Deep sequencing technologies significantly affect the way modern genetic tests are performed. The availability of genome-wide screening technologies shifted the paradigm from few) gene investigation strategies to a multi genes / complete genome analysis. Nevertheless, clinical application are often still focused on the analysis of specific target regions. As a result, a new trend is emerging that exploits the genome-scale approach by “sequencing everything”, but analyzing only the regions of interest of specific diagnostic cases (e.g., diseases). As a consequence, sequencing data that is available, but has not been analyzed, will be reused in the future to answer different questions over time. Moreover, an increasing need for decision support in routine diagnostics with multiple applications will be noticed.

**Methods:** Following this trend, Platomics proposes an analogue approach to software solutions designed for genetic data analysis. The computational approach can be summarized as: one specific software app for each diagnostic use case. This also marks a paradigmatic shift in software development, as it is focused on atomic validated reproducible applications rather than omni-comprehensive highly configurable solutions. Therefore, each application needs to be in line with the specific genetic screening requirements, in terms of ethics, benefits for the patient, and effects on health care costs. **Conclusions:** Genetic-test manufacturers will be enabled to bring their new products faster to the market and lowering the barrier-to-entry by shortening the time-to-market path. Furthermore, the new Platomics approach will provide a streamlined way to drive the adoption of new genetic tests in routine diagnostics.

#### P14.013

##### Carrier risk estimation using SNP-based measures of relatedness

R. Shraga, M. Gold, A. Manoharan, S. Yarnall;  
Recombine, New York, NY, United States.

**Background:** Our goal was to evaluate localized genetic relatedness in clinically-relevant regions. We focused on DNA surrounding genes related to Cystic Fibrosis, Sickle-Cell Anemia, and Smith-Lemli-Opitz Syndrome.

**Methods:** We created three cohorts by selecting carriers of the disorders and adding randomized control patients (non-carriers) of the same ethnicity. To study the relatedness of each group, we selected single nucleotide polymorphisms (SNPs) in the region of the genes implicated in each disease. We created genetic similarity matrices for each cohort using six SNP-based measures of relatedness. We compared average relatedness within the case groups, within the control groups, and between the two groups. This led to development of an initial carrier risk classifier for Cystic Fibrosis mutation DeltaF508, which modifies the k-Nearest Neighbor (KNN) algorithm to use our SNP-based relatedness measure instead of Euclidean distance. We tested the performance of our classifier using repeated random sub-sampling cross-validation.

**Results:** Carriers of all 3 disorders appeared more similar to each other than to controls. We found that inter-group relatedness between sets of patients that are carriers of different mutations for the same disease varied widely. Using the modified KNN classifier, we randomly sub-sampled our cohort into training and testing sets, and found 98% of cases get classified as at risk compared to 20% of controls.

**Conclusion:** These results indicate it may be feasible to classify carrier risk when direct observation of a mutation is not possible. This may be useful for improving sensitivity where direct testing is difficult or error prone.

#### P14.014

##### Variability in apoptosis patterns in cfDNA in body fluids in healthy individuals

B. Gudmundsson<sup>1,2,3</sup>, H. G. Thormar<sup>1,3</sup>, O. Hammarlund<sup>1</sup>, J. Lindblad<sup>1</sup>, M. L. Sigurdardottir<sup>1,2</sup>, S. Rafnssdottir<sup>1,3</sup>, A. Sigurdsson<sup>1</sup>, B. Thorsteinsdottir<sup>1,2</sup>, J. J. Jonsson<sup>1,2</sup>,  
<sup>1</sup>Univ. of Iceland, Reykjavik, Iceland, <sup>2</sup>Landspitali - National University Hospital of Iceland, Reykjavik, Iceland, <sup>3</sup>Lifeind ehf, Reykjavik, Iceland.

**Introduction:** Cell-free DNA (cfDNA) in plasma is used for fetal sexing, NIPT and Rhesus genotyping. cfDNA also holds promise in detecting acquired somatic changes in cancer. We used a new technique, Northern Lights Assay (NLA), to further investigate structure of cfDNA in body fluids in healthy subjects.

**Materials and Methods:** NLA is based on Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE), a technique of nucleic acid separation based on size, strandness, and conformation changes induced by damage. Each specimen is analyzed in sample pairs of non-digested DNA to detect single- and double-stranded breaks and MboI-digested DNA to detect various other lesions. NLA is run in microgel to improve sensitivity. We tested NLA on cfDNA isolated with gentle methods from whole blood, plasma, saliva, urine sediment and cell-free urine in healthy controls (7 males and 13

females) age 21 to 80.

**Results:** Yield from clinical volume samples was sufficient for sensitive analysis with NLA. Variable but generally extensive damage was detected in cfDNA from various body fluids. cfDNA in plasma ranged from essentially at least 3 kb long fragments only to a substantial fraction comprising apoptosis DNA fragments.

**Conclusions:** The findings have implications for cfDNA assays. Consistent differences in rates of apoptosis in healthy subjects seem unlikely. A more plausible explanation is time-coordinated pulse release of cfDNA fragments from apoptotic cells. This pulse would have previously gone undetected using protein markers of cell death with half life of hours in plasma instead of minutes for cfDNA.

#### P14.015

##### Detecting EGFR and related mutations in the cfDNA of lung cancer patients

M. Tamm<sup>1</sup>, K. Oselin<sup>2</sup>, P. A. Kivistik<sup>1</sup>, K. Keerma<sup>1</sup>, R. Roosipuu<sup>3</sup>, M. Saare<sup>1</sup>, H. Roomere<sup>4</sup>, A. Metspalu<sup>1,5</sup>, N. Tõnnisson<sup>1,4</sup>,  
<sup>1</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>2</sup>North Estonia Medical Centre, Tallinn, Estonia, <sup>3</sup>Dept. of Pathology, Tartu University Hospital, Tartu, Estonia,  
<sup>4</sup>Dept. of Genetics, Tartu University Hospital, Tartu, Estonia, <sup>5</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

**Introduction:** According to WHO, lung cancer is the leading cause of cancer deaths worldwide. Besides invasive tumor biopsy, molecular diagnostic analysis of tumor cell-free DNA is becoming increasingly popular as a method enabling to capture the whole tumour heterogeneity in almost real-time setting. This may have applications in both early diagnostics, as well as therapeutic monitoring of alterations predictive for drug response.

**Objectives, materials, methods:** Our primary objective was to set up a straightforward platform for the analysis of clinically relevant mutations. Our study cohort currently comprises of 58 lung adenocarcinoma patients who have donated blood plasma samples prior to initiation of chemo- or targeted therapy. The cohort will be longitudinally monitored and repeated blood samples collected upon progression. FFPE tumor samples have been available for approximately 50% of the study subjects. We have currently set up allele-specific fragment analysis workflow for EGFR gene common mutations and amplicon-based multiplex next-generation sequencing (NGS) in 5 lung cancer-related genes (EGFR, BRAF, HER2, KRAS, PIK3CA).

**Results:** Our preliminary results show that both approaches detect <1% mutant allele content. The methods will be compared in both cfDNA and FFPE analysis, as well as with the available clinical DNA data.

**Perspectives:** Our further aim is to collect longitudinal samples and perform a larger screen of drug susceptibility and resistance mutations in our study cohort, involving detection of point mutations, copy number analyses and DNA methylation changes. We strongly believe that cfDNA analysis will become a clinical routine in various cancers in the near future.

#### P14.016

##### A low cost, comprehensive, ctDNA assay based on targeted enrichment of rare alleles and error-reduced sequencing

D. Broemeling<sup>1</sup>, J. Pel<sup>1</sup>, M. Wiggin<sup>1</sup>, A. Marziali<sup>1,2</sup>,

<sup>1</sup>Boreal Genomics, Vancouver, BC, Canada, <sup>2</sup>University of British Columbia, Vancouver, BC, Canada.

Identification of circulating tumour DNA in a blood sample (liquid biopsy) can be used to detect and genetically profile tumours in a routine and minimally invasive assay. As certain somatic mutations are specifically associated with cancer, liquid biopsy has the potential to be an exceptionally specific and low-risk screening tool that could stage-shift cancer detection to earlier, more curable stages.

We present two complementary methods to optimize sensitivity over broad gene regions at low cost. We combine a highly selective enrichment technology to screen hotspot mutations at very high sensitivity, with a high accuracy sequencing technology that can scan large regions of the genome at low cost, albeit with reduced sensitivity. Using the commercially available OnTarget enrichment technology, activating mutations consisting of SNVs and short indels can be preferentially selected for sequencing over wild-type sequence. This approach enables highly sensitive detection to 0.01%, or below, for up to 1,000 unique variants.

Broader coverage of regions without hotspot mutations, as well as detection of copy number variants and translocations, can then be addressed with a novel library construction process for NGS sequencing that increases the accuracy of the workflow by an order of magnitude. Named Proximity-Sequencing (Pro-Seq), the method duplicates sequence information in each original DNA strand, achieving sensitivity in the 0.1-0.01% range depending

on read depth.

The addition of Pro-Seq library construction to OnTarget enrichment enables low cost, high sensitivity, liquid biopsy tests to be developed, enabling commercialization in applications with limited reimbursement, potentially including early cancer detection.

#### P14.017

#### The difficult interpretation of blood mosaicism in routine diagnostic specimens: discordant results between conventional cytogenetic/FISH and CGH array

*A. Plaja, 08035, T. Vendrell, N. Castells, A. Cueto-González, M. Borregan, F. López-Grondona, L. Martínez-Ribot, Q. Ferrer-i-Carbonell, E. Tizzano; Dept. Clinical and Molecular Genetics. H. Vall d'Hebron, Barcelona, Spain.*

**Introduction:** Low-level somatic mosaics can be associated with severe birth defects, but reliable detection remains challenging. We describe three cases with discordant cytogenetic/FISH and CGH array results in blood samples.

#### Material and methods:

Patient 1: aged 14 years, shows epileptic encephalopathy and a normal 46,XY[15] karyotype.

Patient 2: aged 32 years, shows Fallot tetralogy and leukopenia.

Patient 3: aged 4 years, shows global developmental delay and karyotype result of 46,XY, del(18)(q21.3)[13]/46,XY[17]

All patients were studied with array CGH (Agilent, USA, G4827A CGH ISCA v2, 8x60K) and Cytogenomics CNV detection software (Agilent, USA). Cytogenetic techniques involved G banding and FISH with BACs RP11-81j7 (1p36.22) and RP11-81h19 (1q24).

**Results:** Patient 1: CGH-array Cytogenomic software (CS) showed a 1q21.1q32.1 mosaic duplication (hg19: 145415190-206794531). A new chromosomal study revealed a 46,XY, dup(1)(q21q32.1)[4]/46,XY[331] and FISH analysis revealed two nucleus and one metaphase of 300 showing 3 signals.

Patient 2: CGH-array CS showed a 1p36.32p36.13 mosaic deletion (hg19: 5159991-17753699) and FISH analysis revealed 23 nucleus of 233 showing only one signal.

Patient 3: CGH-array CS did not reveal CNVs in chromosome 18. Visual inspection of CS plots revealed an 18q21.31q22.2 mosaic deletion (54370433-68956470).

**Conclusions:** CGH-array analysis was able to detect mosaic CNVs of 1.2% (1q21dup) and 9.9% (1p36del), but failed to detect the presence of a 43% 18q21.31q22.2 mosaic deletion.

CGH-array analyzes DNA from all nucleated blood cells while cytogenetic/FISH on cultured samples analyzes only T-lymphocytes. Our results highlight the importance of applying combined molecular and cytogenetic techniques when there is suspicion of mosaic anomalies.

(FIS:PS09/00632)

#### P14.018

#### four cases of CNVs identification using an exome sequencing approach

*P. Maietta<sup>1</sup>, J. Botet<sup>1</sup>, M. Peña-Vilabesta<sup>1</sup>, M. Martínez-García<sup>1</sup>, D. Martínez Fernández-Mayoralas<sup>2</sup>, L. Martorell Sampol<sup>3</sup>, A. Fernández-Perrone<sup>2</sup>, S. Álvarez de Andrés<sup>1</sup>;  
<sup>1</sup>NIMGenetics, Madrid, Spain, <sup>2</sup>Universitary hospital Quirónsalud, Madrid, Spain, <sup>3</sup>Universitary children's hospital Sant Joan de Deu, Barcelona, Spain.*

**Introduction:** For the identification of molecular defects in patients with suspected genetic disorders, whole exome sequencing (WES) has now entered in medical practice as a diagnostic approach. Unfortunately, it presents some limitations for CNVs detection, principally due to the unbalanced distribution of the exons through the different chromosomes.

**Material and Methods:** We performed exome sequencing using the Ion AmpliSeqTM exome technology (Life Technologies) with Ion ProtonTM and IonS5-XLTM. Sequencing reads were analysed using Ion ReporterTM Software, where the overall distribution and depth has been normalized against a baseline generated with data coming from 10 unrelated males.

**Results:** Here we present 4 cases with phenotypes associated to 4 different disorders, such as epilepsy, spastic paraplegia, psychomotor retardation and intellectual disability. Using the score generated by the automatic software, in combination with the clinical information, we were able to prioritize deletions of different sizes ranging from 5kb on PLP1 gene to 5Mb on the long arm of chromosome 2. These alterations were posteriorly validated using array CGH technology or MLPA. The criteria used for the identification on those cases will be presented.

**Conclusions:** WES presents different limitations for the detection of CNV, but for some regions the data can be used to suggest the presence of regions with aberrant copy changes. The systematic evaluation of this information could help to accelerate the patient's diagnosis.

#### P14.019

#### Application of CNV Sequencing for whole genome copy number variant detection over a two year period

*P. Roberts<sup>1</sup>, S. V. Hewitt<sup>1</sup>, A. Coates<sup>1</sup>, C. M. Watson<sup>1,2</sup>, L. Crinnion<sup>1,2</sup>, D. T. Bonthron<sup>2</sup>;*

*<sup>1</sup>Leeds Genetics Lab, St James's Hospital, Leeds, United Kingdom, <sup>2</sup>School of Medicine, St James's Hospital, Leeds, United Kingdom.*

**Introduction:** Since January 2014 we have applied CNV Sequencing, a next generation sequencing technology, to assess DNA dosage status either to validate array CGH findings, or for poor quality/low volume samples that fail the QC status for array CGH.

**Materials and Methods:** The technique uses the Illumina HiSeq 2500 Sequencing platform for whole genome copy number variant detection and relies on a custom-designed computational pipeline for copy number variant calling. Ten samples are run in a multiplex, each analysis being based on 20-25 million single-end 50-nt reads.

**Results:** From 2014-2016, we have sequenced over 220 diagnostic samples from difference sources, i.e. blood, tissue from products of conception, amniotic fluid, saliva. A successful result was obtained for 206/224 samples, with an average resolution per patient of 40 kb, and an analytical sensitivity of ~98% for imbalances ≥40 kb. Higher resolution can be achieved by sequencing the libraries at higher coverage with relatively minimal cost implications.

**Conclusions:** CNV Sequencing leads to improved success rates on low volume/poor quality DNA samples, and presents additional advantages over array CGH via its digital approach to evaluating DNA dosage changes. We are presently evaluating the potential for its introduction in prenatal diagnosis where suboptimal DNA quality for array CGH is especially problematic. We believe that our method is cost effective, reliable and accurate and that it potentially overcomes the technical challenges of calling dosage changes by whole exome sequencing.

#### P14.020

#### Detecting copy number variation by limiting dNTP PCR and high-resolution melting

*L. Zhou<sup>1</sup>, R. Palais<sup>2</sup>, C. Wittwer<sup>1</sup>;*

*<sup>1</sup>Department of Pathology, University of Utah School of Medicine, Salt lake City, UT, United States, <sup>2</sup>Department of Mathematics, Utah Valley University, Orem, UT, United States.*

**Introduction:** About 13% of genes in the human genome have variation in copy number. Copy number alterations (CNAs), somatic changes to chromosome structure that result in gain or loss in copies of sections of DNA, are very common in cancer and associated with particular cancer types.

**Materials and Methods:** Ratios of reference and copy number variants were maintained by limiting quantities of dNTPs and allowing PCR to plateau. Suitable fragments having single melting domains, well-separated Tms, and no common homologs were designed using uMelt melting curve prediction software. MeltWizard 6 software was used to remove background, equalize negative derivative melting peaks corresponding to the reference segment, and identify and quantify samples with different CNV ratios from the amplitude of their target melting peaks.

**Results:** CNV ratios far beyond this range, as small as 2: 2.125 (5.88%), were detectable using the most robust, simplest, and highest resolution method of limiting dNTPs at an optimal 3.25 uM. Duplex PCR of reference and a target was used to detect copy number variation in SMN1, SMN2, EGFR, chromosome X, Y, 13, 18, and 21. Blinded test of 50 potential trisomic samples were concordant to karyotyping. In 7 out of 8 of lung cancer, the copy number of EGFR is higher than normal copy 2.

**Conclusion:** The simple closed-tube method is fast, economical, more accurate and less susceptible to contamination than other methods. Assays are easy to design, limiting dNTPs is simple, and the results are stable and reliable.

#### P14.021

#### CoNVaDING: single exon variation detection in targeted NGS data

*F. van Dijk, L. F. Johansson, E. N. de Boer, K. K. van Dijk-Bos, J. D. Jongbloed, A. H. van der Hout, H. Westers, R. J. Sinke, M. A. Swertz, B. Sikkema-Raddatz;*

*Department of Genetics, University Medical Center Groningen, Groningen, Netherlands.*

We have developed a tool for detecting single exon copy number variations (CNVs) in targeted next-generation sequencing data: CoNVaDING (Copy Number Variation Detection In Next-generation sequencing Gene panels). Existing methods consider all control samples equally informative even though there are sample to sample variations caused by differences in PCR and capturing efficiency. CoNVaDING selects the control samples showing a coverage pattern most similar to that of the sample analysed. Data is

then normalized, using within the sample all autosomal targets or all targets within the same gene. Based on the normalized data, for each target the ratio of the normalized average read depth of the sample to that of the controls and a distribution analysis using a Z-score are calculated. Based on the calculated ratio and distributions a prediction is made for each target to determine whether a CNV is present or not, providing additional sample quality metrics during several stages of the analysis.

We compared the performance of CoNVaDING with XHMM and CoNIFER in 320 samples captured with two different targeted gene-panels containing in total 308,574 exons. For all CNV calls made by one of the three methods MLPA was performed. CoNVaDING detected all known CNVs in high quality targets, giving 100% sensitivity, at a 99.998% specificity. Thereby outperforming XHMM and CoNIFER by exhibiting a higher sensitivity and specificity and by precisely identifying low-quality samples and regions. These improved quality control metrics enable use in both research and clinical diagnostics setting.

#### P14.022

#### Evaluation of CNV detection from targeted next-generation panel sequencing data in routine diagnostics

A. M. Nissen, M. Locher, C. Rapp, A. Laner, A. Benet-Pages, E. Holinski-Feder; MGZ Medical Genetics Center, Munich, Germany.

Copy number variation (CNV) detection from exon-capture NGS data is challenging because of non-uniform capture efficiency of targeted exons or short read lengths. Often CNV detection in routine diagnostics is still performed by additional techniques (MLPA, microarray) instead of using NGS data for both SNV and CNV. We used a bioinformatics pipeline comprised of ExomeDepth, XHMM and in-house methods to detect exonic CNVs using read depth data derived from a targeted NGS Panel (Illumina TruSight Cancer). Capture efficiency per exon was assessed with a set of CNV negative patients to identify reliable regions. Additionally, genes with pseudogenes, paralogues and repeat regions were excluded from the study design. We present a first evaluation with a set of 86 patients previously tested for SNVs (NGS) and CNVs (MLPA). 51 patients presented pathogenic SNVs and 35 patients pathogenic CNVs. We confirmed 15 deletions and two duplications with high significance. Another nine deletions and two duplications were correctly detected with lower confidence, which affected a smaller number of exons compared to the high confidence results. In addition, five inconspicuous cases showed contradictory results due to SNVs affecting MLPA-probe binding site. In two cases deletion events of one exon could not be detected. No additional CNVs were found in patients with pathogenic single mutations. This proof-of-concept study demonstrates the feasibility of using a single testing strategy to detect simultaneously SNVs and CNVs for clinical diagnosis. However, this method is only applicable for selected genes and consistent workflow conditions.

#### P14.023

#### Whole-gene CFTR sequencing combined with digital RT-PCR improves genetic diagnosis of cystic fibrosis

L. Straniero<sup>1,2</sup>, G. Soldà<sup>3,2</sup>, L. Costantino<sup>4</sup>, M. Seia<sup>4</sup>, P. Melotti<sup>5</sup>, C. Colombo<sup>6</sup>, R. Aselta<sup>3,2</sup>, S. Duga<sup>3,2</sup>;

<sup>1</sup>Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy, <sup>2</sup>Humanitas Clinical and Research Center, Rozzano-Milano, Italy,

<sup>3</sup>Department of Biomedical Sciences, Humanitas University, Rozzano-Milano, Italy,

<sup>4</sup>Medical Genetics Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>5</sup>Cystic Fibrosis Center, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy, <sup>6</sup>Cystic Fibrosis Center of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

**Introduction:** Cystic fibrosis (CF) is a common recessive disorder caused by >1,900 mutations in the CFTR gene. Despite extensive screening, 1-5% of patients lack a definite molecular diagnosis. Here, we propose an efficient, sensitive, and cost-effective whole-gene NGS protocol applied to the molecular screening of CF patients.

**Methods:** We designed a custom Nimblegen SeqCap EZ capture kit targeting the entire 189-kb-long CFTR gene and the coding portions of the SCNN1A, SCNN1B, and SCNN1G genes, encoding the subunits of the sodium channel ENaC, reported to be mutated in CF-like patients. Sequencing was performed on an HiSeq2000 platform, multiplexing up to 18 samples in a sequencing lane.

**Results:** We analyzed 23 CF patients and one carrier: 4 previously-characterized patients served as controls; 17 were lacking a complete diagnosis after conventional CFTR screening; 3 were not previously genetically screened. We obtained a mean depth >1,150X and 98% coverage of the target region. Our approach allowed the identification of 22 previously-known CFTR mu-

tations, including 2 large deletions. For 2 patients, compound heterozygous for a CFTR mutation and the intron-9 c.1210-34TG[11-12]T5 allele - associated with decreased CFTR mRNA levels - the molecular diagnosis was implemented by measuring the residual level of wild-type transcript by digital RT-PCR on RNA extracted from nasal brushing.

**Conclusions:** We demonstrated that whole-gene resequencing combined with digital RT-PCR is a cost-effective and accurate approach for the genetic testing of CF and CF-related disorders.

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#### P14.024

#### Compiling a default list for intellectual disability in different laboratories in the Netherlands

M. Kriek<sup>1</sup>, C. A. L. Ruivenkamp<sup>2</sup>, K. L. I. van Gassen<sup>3</sup>, M. A. van Slegtenhorst<sup>4</sup>, A. P. A. Stegmann<sup>5</sup>, R. P. Pfundt<sup>6</sup>, T. Dijkhuizen<sup>7</sup>, M. Alders<sup>8</sup>, M. M. Weiss<sup>9</sup>;

<sup>1</sup>Department of Human and Clinical genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>2</sup>Department of Clinical genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>Department of Medical Genetics, University Medical Center, Utrecht, Netherlands, <sup>4</sup>Department of Clinical Genetics, Erasmus MC, Rotterdam, Netherlands,

<sup>5</sup>Department of Clinical Genetics, Maastricht UMC, Maastricht, Netherlands,

<sup>6</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, <sup>7</sup>Department of Clinical Genetics, University Medical Center, Groningen, Netherlands, <sup>8</sup>Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands, <sup>9</sup>Department of Clinical Genetics, VU Medical Center, Amsterdam, Netherlands.

Using exome sequencing, the coding genetic material (exons) is examined for genetic variants. In accordance with recent guidelines abnormalities will initially be sought in a dedicated list of genes (default list) with a known relationship to the patient's disease (targeted analysis). Our goal was to get an insight of what choices were made regarding the content of the list with dedicated genes in different laboratories in the Netherlands. A questionnaire was sent out in 2015 to all (n=7) clinical genetic laboratories in the Netherlands after one contact person per laboratory had agreed to participate in this study. Six laboratories returned the questionnaire. It contained 10 questions regarding the selection of genes for the default list of intellectual disability.

The process of compiling the list is dissimilar on a quite a number of items; there were discrepancies in both the experts involved as the sources used between the laboratories. In addition, the level of evidence for causality e.g. selection criteria for genes, differed slightly between laboratories. Points of internal discussion included the application of genes present in the heel prick and whether the DMD- and TTN gene should be added to the list. The agreements included the frequency of updating the list and the willingness of sharing its content. The combined questionnaire results will help to set standards to the selection process of genes for default lists and may lead to a national agreement on a minimal list of core genes (e.g. genes that are covered completely) for intellectual disability.

#### P14.025

#### The 100,000 Genomes Project: Improving DNA quality, it's in our blood

N. Iley<sup>1</sup>, V. Davison<sup>2,3</sup>, Z. Deans<sup>1</sup>, S. Hill<sup>2,3</sup>,

<sup>1</sup>UK NEQAS for Molecular Genetics, Edinburgh, United Kingdom, <sup>2</sup>NHS England, London, United Kingdom, <sup>3</sup>Health Education England, London, United Kingdom.

Whole genome sequencing (WGS) requires the input of genomic DNA of sufficient quantity and quality to ensure a high quality sequencing output. To ensure samples submitted into England's 100,000 Genomes Project are of appropriate quality to enable accurate WGS, NHS England with UK NEQAS for Molecular Genetics have developed an external quality assessment (EQA) scheme for the extraction of high quality genomic DNA from fresh whole-blood samples. All laboratories providing DNA samples for inclusion in the project are required to participate in this EQA scheme.

Since December 2014, laboratories have participated in three practical EQA runs. In each run, three homogenised donor whole-blood samples of varying volumes were provided and laboratories instructed to extract DNA from the whole sample and return it to UK NEQAS for analysis. The quality metrics assessed and scored include DNA sample volume, total DNA mass, 260/280 ratio, Illumina Delta Cq assay, Library Preparation and Bioanalyzer trace using the Trusight Cancer Panel and agarose gel electrophoresis. An overall combined score for genomic DNA quality was generated and laboratories bench-marked against each other.

Initial results showed a broad range of DNA extraction methodologies and a high variation in DNA quality. Continued participation in EQA runs showed marked improvement in the quality of DNA extracted.

Participation in DNA extraction EQAs highlighted extraction methodologies used for routine molecular genetics applications were not entirely suitable for WGS. Application of quality metrics, inter-laboratory bench-marking and continued participation allowed laboratories to identify areas of concern promptly and address them efficiently and effectively.

#### P14.027

#### PepPipe and MethPipe: pipelines for discovery and validation of DNA-methylation and antibody profiles to improve hereditary autoinflammatory disease diagnosis

*A. Weinhaeusel, Manuela Hofner, Walter Pulverer, Stephan Pabinger, Regina Soldo, Peter Hettetegger, Ram Vinay Pandev, Lisa Milchram, Ronald Kulovics, Silvia Schönthal, Gabriel Beikircher, Christa Noehammer, Clemens Vierlinger; AIT, Vienna, Austria.*

Systemic autoinflammatory diseases (SAID) are highly heterogeneous monogenic or multifactorial conditions secondary to deregulation of mechanisms controlling the innate immune response. Best known examples are the hereditary periodic fever-syndromes; however molecular analysis is inconclusive in 70-80% of "undefined SAID" patients. Thus there is a great need to improve correct diagnosis. Within the recently started E-Rare-3 project "INSAID", the AIT Molecular Diagnostics group will use their analysis pipelines MethPipe and PepPipe to perform highly paralleled epigenetic and immunomic profiling of Familial Mediterranean Fever (FMF) as a model. Here we will present our two pipelines:

PepPipe: Protein-microarrays presenting about 7400 different human proteins from 15284 expression clones and high density peptide arrays are used for highly efficient auto-antibody profiling. For validation we have established Luminex-assays enabling high-multiplexed targeted analyses of antigenic markers. In parallel immune-qPCRs will be performed for 92 inflammation markers from 1µl serum using Proseek® Multiplex assays.

MethPipe: Genome-wide methylation screening is performed via Illumina's 850k arrays which can be combined with targeted bisulfite sequencing for targeted analysis. Highly paralleled marker validation is then carried out via methylation-sensitive restriction enzyme coupled MSRE-qPCR using Fluidigm's Biomark system, enabling testing up to 96 samples and 96 assays in a single qPCR run.

The findings of these experiments will be used to characterize undefined SAID on the epigenetic-, immunomics-, and inflammatory- level of molecular disease pathology and to identify new biomarkers to improve correct disease diagnosis.

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#### P14.028

#### Novel PCA-based normalization technique for inferring exon level copy number

*M. Gold, R. Shraga, S. Yarnall; Recombine, New York, NY, United States.*

Background: Most microarray-based CNV-detection algorithms infer copy number by comparing a bead's intensity for a given sample to a control intensity (called an r-ratio). We have developed a novel normalization technique to improve exon-level CNV-calling and tested it on the DMD gene.

Methods: We used the Illumina Infinium Assay with custom content and designed unique beads to cover the DMD exons and the fifty nucleotides surrounding each exon. We applied Principal Component Analysis (PCA) to the r-ratio for 2671 beads with low minor allele frequency outside of the DMD gene in an attempt to capture intensity variability due only to technical noise. Copy number was determined by comparing a sample's observed r-ratio per exon to the r-ratio predicted for that exon by a regularized linear regression model trained on the principal components of the outside beads.

Results: We ran the algorithms on 1715 female research-consented samples, using a null-call threshold based on the smoothness of the r-ratio values across exons. We observed 733 normal, 976 NC, and 6 CNV calls for the standard r-ratio algorithm and 1698 normal, 15 NC, and 2 CNV calls for the novel algorithm.

Discussion: We validated the two PCA-normalized CNV calls using an outside lab. Patterns in the four CNV calls made by the un-normalized algorithm indicate false positive calls, but this was not confirmed. This novel technique further normalizes r-ratio data by removing non-biological noise and can be used to improve exon-level copy number detection for screening and diagnostic microarray assays.

#### P14.029

#### Multiplex TaqMan Assays for Rare Mutation Analysis Using Digital PCR

*M. Laig, F. Chan, L. Lac, B. Moy, T. Straub, K. Varma, D. Keys; Thermo Fisher Scientific, South San Francisco, CA, United States.*

Introduction: Detection of rare mutations for research purposes in tumor tissue and cell free DNA (cfDNA) allows for monitoring of tumor progression and regression. cfDNA isolated from plasma combined with a sensitive detection method like digital PCR is non-invasive and enables earlier detection compared to conventional imaging techniques.

Building on the TaqMan based Rare Mutation assay set for detection of rare mutations using digital PCR on the QuantStudio 3D Digital PCR System, we are now developing multiplex assays for simultaneous detection of several mutations. We selected relevant mutations in the EGFR and KRAS genes for our initial multiplex application: EGFR G719, EGFR exon 19 deletions, and KRAS G12/G13. These mutations may have implications for potential future targeted therapy.

Methods: Primers and probes of singleplex Rare Mutation Assays were reformulated to generate multiplex assays detecting the EGFR and KRAS mutations. All multiplex assays were tested on template composed of wild-type genomic DNA background mixed with mutant plasmid reflecting each of the mutations detected by the multiplex assays.

Summary: Initial experimental results were successful and showed excellent signal intensity and clear cluster separation when analyzed with the QuantStudio 3D AnalysisSuite™ Cloud Software. The EGFR G719 mutations (COSM6239, COSM6253, COSM6252) were detected using a 3plex assay, EGFR exon 19 deletions (COSM12383, COSM12422, COSM12678, COSM6223, COSM6254, COSM6255) were detected using a 6plex assay, and KRAS G12/G13 mutations are underway.

Conclusion: Multiplexing assays for three relevant mutation loci proved feasible and presents an efficient way to assess the presence and the percentage of mutations.

#### P14.030

#### Phenotyping Emanuel Syndrome using computer-aided facial dysmorphology analysis of 2D photos

*A. B. Hamid Al-Rikabi<sup>1</sup>, N. Fleischer<sup>2</sup>, M. Rinholm<sup>3</sup>, S. St-Pierre<sup>3</sup>, I. Schreyer<sup>1,4</sup>, T. Liehr<sup>1</sup>; <sup>1</sup>Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany, <sup>2</sup>FDNA, Herzliya, Israel, <sup>3</sup>Chromosome 22 Central, Fuquay-Varina, NC, United States, <sup>4</sup>Center for Ambulant Medicine, Human Genetics, Jena, Germany.*

While sequencing technology and variant interpretation are continuously progressing, the critical process of phenotyping and gathering clinical information has not changed much. Here the facial dysmorphology novel analysis (FDNA) technology was used to automatically identify facial phenotypes associated with Emanuel Syndrome (ES; OMIM 609029) based on 2D facial photos. ES, also denominated supernumerary der(22)t(11;22) syndrome is characterized cytogenetically by a small supernumerary marker chromosome (sSMC). Clinical signs include severe mental development delay, microcephaly and dysmorphic features. Besides heart defects, cleft lip and palate, kidney malformations and anal atresia, etc., can complicate the clinical problems. Here 65 facial photos of molecularly diagnosed ES patients were compared to unaffected controls (n=1000) and to a second control group (n=1000) of individuals affected with one of 100 other syndromes with facial phenotypes. The mean area under the curve (AUC) comparison between ES and normal individuals (0.99, STD 0.001) expresses an almost full separation between these two cohorts. A slightly lower mean AUC (0.985, STD 0.046) was obtained when comparing between ES and individuals affected with other syndromes. Our preliminary results show that computer aided facial recognition is able to help in the clinic and could possibly reduce the time patients spent in the diagnostic odyssey. Also it may help to differentiate ES from other patients with sSMC, especially in countries with no access to more sophisticated genetic approaches apart from banding cytogenetics. Inclusion of more facial pictures of ES-patient is welcome and may also contribute to a higher detection rate.

#### P14.031

#### Detection method for the 3'EPCAM genomic deletion and its frequency in Polish HNPCC patients

*P. Borun<sup>1</sup>, J. Szczepinska<sup>1</sup>, M. Siolek<sup>2</sup>, B. Kozak-Klonowska<sup>2</sup>, A. Plawski<sup>1</sup>;*

*<sup>1</sup>Institute of Human Genetics, Poznan, Poland, <sup>2</sup>Department of Genetics, Holycross Cancer Centre, Kielce, Poland.*

Lynch syndrome is a frequent, autosomal, dominantly-inherited cancer predisposition caused by various germline alterations that affect DNA mismatch repair genes, mainly MLH1, MSH2, MSH6 and PMS2. Large rearrangements of the EPCAM (Epithelial Cell Adhesion Molecule) gene, which is localized

on chromosome 2 upstream of the MSH2 gene, have been recently described as a genetic cause of the Lynch syndrome occurrence. The rearrangements, encompassing mainly 3' end of the EPCAM gene, lead to mismatch repair deficiency in some Lynch syndrome families.

The aim of the study was to develop cost-effective screening tool for the rapid detection of 3'EPCAM genomic rearrangements, along with its validation, and determination of the 3'EPCAM mutation status in our group of polish HNPCC patients. We applied C-HRM, a method enabling the detection of 3'EPCAM rearrangements and simultaneously screening for small mutation in two exons of the MLH1 gene containing small mutation hot-spots. With the developed assay, in our group of 250 Lynch syndrome probants, we detected 2 cases of 3'EPCAM genomic rearrangement and 4 small mutations within the studied exons of the MLH1 gene.

#### P14.032

##### An exome routine genetic diagnoses assay

*B. Cogne, P. Boisseau, S. Schmitt, S. Kury, X. Latypova, W. Deb, J. Le Gall, M. Nizon, M. Vincent, T. Besnard, S. Mercier, B. Isidor, S. Bezieau; CHU Hotel Dieu, Nantes, France.*

**Background and aims:** As shown by the abundance of publications, exome may be a promising technique to discover genes causing diseases. But, could exome be an efficient routine strategy to perform genetic diagnoses? We wanted to ask this question in current convergence of some difficult pathology presentations and an easy access to exome technology.

**Method:** We selected 20 patients affected with greatly heterogeneous genetic pathologies without any obvious gene causing disease or previous unsuccessful targeted genes analyses. Exome sequencing was done for each of them. We analysed raw data with an in-house bioinformatic pipeline "phenotype guided" designed (it uses HP terms for the pathology description which fit or not with Human Phenotype Ontology database), the filter used was 1000g database. This pipeline generated a set of sequence variations according to possible mode of inheritance and MIM data. Candidate sequence variations were confirmed by Sanger sequencing and family segregations.

**Results:** The first results were encouraging: 11 genes were very likely pathogenic (55% of the patients) and 4 others were possibly deleterious (20%).

**Discussion and conclusions:** These first results allow us to continue this assay to confirm or not these promising test performances. Nevertheless, improvements have to be implemented to this strategy to avoid missdetection of sequence variations, like the use of a SNP local database, a bioinformatic splice testing for silencing SNP and exon-intron junction nucleotides and an analysis of copy number variations.

#### P14.034

##### MedExome: An optimized whole exome design for identification of medically relevant genetic variants

*M. Brockman, H. Rosenbaum, L. Lincoln, D. Burgess, T. Richmond, J. Wendt; Roche NimbleGen, Madison, WI, United States.*

**Introduction:** Whole exome sequencing provides a comprehensive view of protein coding regions, but the typical depth of sequencing can result in regions of low or missing coverage throughout the exome. Mitochondrial variant calling is also of increasing interest but has been difficult to combine with exome enrichment. Here we present performance of MedExome, an optimized whole exome sequencing design with the option for including mitochondrial target enrichment in a streamlined workflow.

**Materials and Methods:** 100ng of DNA from samples of varying populations (Coriell Institute; BioServe) was used in library prep from Kapa BioSystems. Sequence capture with multiple iterations of Roche NimbleGen SeqCap EZ MedExome with overnight hybridization was used to gather empirical data and optimize capture design performance. Sequencing at 2x101bp was performed on Illumina HiSeq 2500 instruments.

**Results:** We used empirical data at multiple stages of development to optimize performance resulting in high uniformity (fold 80 base penalty 1.90-2.03) and an average of 95.7% of bases with 20X coverage or higher given 6Gb of raw sequencing. We developed a companion protocol for optional enrichment of the mitochondrial genome without sacrificing whole exome coverage.

**Conclusions:** We have developed MedExome, a new whole exome sequencing design with application for the identification of known and novel medically relevant variants with the option of combining mitochondrial and whole exome sequencing.

For Life Science Research Use Only. Not for use in diagnostic procedures. This study was supported by Roche NimbleGen, a part of Roche Sequencing. All authors are employees of Roche NimbleGen.

#### P14.035

##### Clinical whole-exome sequencing for the diagnosis of rare disorders with multiple congenital anomalies: feedback on 400 consecutive tests

*J. Thevenon<sup>1</sup>, S. Nambot<sup>1</sup>, P. Kuentz<sup>1</sup>, Y. Duffourd<sup>1</sup>, S. El Chehadeh-Djebar<sup>2</sup>, D. Lehalle<sup>1</sup>, N. Jean-Marçais<sup>1</sup>, A. Masurel-Paulet<sup>1</sup>, P. Callier<sup>1</sup>, N. Marle<sup>1</sup>, A. Mosca-Boidron<sup>1</sup>, C. Poë<sup>1</sup>, T. Jouan<sup>1</sup>, M. Chevarin<sup>1</sup>, N. Gigot<sup>1</sup>, A. Bruel<sup>1</sup>, M. Lefebvre<sup>1</sup>, J. Saint-Onge<sup>1</sup>, E. Tisserant<sup>1</sup>, Deleuze<sup>2</sup>, J. Rivière<sup>1</sup>, L. Faivre<sup>1</sup>, C. Thauvin-Robinet<sup>1</sup>;*

*<sup>1</sup>Centre Hospitalier Universitaire, Dijon, France, <sup>2</sup>Centre Hospitalier Universitaire, Strasbourg, France, <sup>3</sup>Centre national de génotypage, Evry, France.*

The World Health Organization estimates the existence of more than 8.000 rare disorders. Each of these disorders is individually rare and affect by definition less than 5/10.000 individual, but are collectively frequent with 8% of the overall population affected. The prevalence of most of rare disorders cannot even be estimated and are ascertained through several publication or case reports. Most of the rare disorders are suspected to be Mendelian diseases with high genetic and clinical heterogeneity. To enhance the diagnostic yield of our generalist consulting genetic center, we have developed a clinical proband whole-exome sequencing strategy. This study reports on the clinical and molecular characterization of a cohort of 400 consecutive proband with multiple congenital anomalies who have had exome sequencing.

Whole-exome sequencing was performed in collaboration with the Centre National de Génotypage. Raw data were analysed on the Computing centre of the University of Burgundy. Overall, the mean depth of coverage was 90x, with 93% of coding regions referenced in RefSeq and sequenced by at least 10 reads. Actually, 297 results were returned to the clinician with a diagnostic yield of 30% for positive results, 15% of non-conclusive results, based on current scientific knowledge. In the first 300 individuals, 2 secondary findings were reported. Twelve months after returning the results, a prospective re-analysis of 130 exomes identified eight additional diagnoses (6%). Pro-active international data-sharing through the Match-Maker exchange initiative allowed the identification of recurrent implication of several new disease-causing genes.

#### P14.036

##### A comparative evaluation of two DNA exome library preparation methods for ion torrent Proton

*P. Gampawar<sup>1</sup>, Y. Saba<sup>1</sup>, M. Bemelmans<sup>2</sup>, J. Semmler-Bruckner<sup>1</sup>, H. Schmidt<sup>1</sup>;*  
*<sup>1</sup>Medical University of Graz, Graz, Austria, <sup>2</sup>University of Applied Sciences. Biomedical Sciences, Graz, Austria.*

##### Aims:

Whole exome sequencing is a cost effective way to detect common and rare variants. While several studies on library preparation methods for Illumina platforms are available, data for the Ion Proton™ sequencer are sparse. Here we compare the performance of AmpliSeq™ Exome RDY IC and SureSelect Human All Exon V6 library preparation kits.

##### Methods:

Genomic DNA was extracted from 4 human blood samples. In total 100 ng of DNA was used for AmpliSeq and 1µg for SureSelect libraries. Template preparation and loading of the chip was done on Ion Chef Instrument. The Ion Reporter™ software was used for data analyses. Validation of variants against microarray data (Affymetrix Human\_SNPArray6.0 and Illumina HumanExome\_v1.1) is in progress.

##### Results:

Parameters	AmpliSeq	SureSelect
DNA input	100ng	1µg
Library preparation time	6 hour	2.5 days
Reference BED file	AmpliSeqExome_20141113 (60.5Mb)	V6-BED_Test.4 (58.72Mb)
Total bases sequenced/sample	7.2Gb	4.4Gb
Number of reads/sample	40278595	40164626
Mean Read length	179bp	130bp
Reads on target	>95%	>80%
Mean coverage	130X	55X
Number of variants/sample	37694.5	45840.25

##### Conclusion:

Both methods showed high level of target enrichment and covered large part of targeted region. AmpliSeq needed lower input DNA and shorter library preparation times. It also showed longer read length and higher mean coverage. SureSelect identified more variants. Validation of variants against GWAs and exome chip data is important to identify false positive and negative calls.

## P14.037

### Validation of a ten-gene panel for improved diagnosis of familial MDS/AML

**G. Ryan<sup>1</sup>, P. Page<sup>1</sup>, S. Akiki<sup>1</sup>, S. R. C. Cardoso<sup>2</sup>, T. Vulliamy<sup>2</sup>, I. Dokal<sup>2</sup>, J. Fitzgibbon<sup>2</sup>, Y. Wallis<sup>1</sup>**

<sup>1</sup>West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital, Birmingham, United Kingdom, <sup>2</sup>Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom.

Cases of familial Myelodysplastic Syndrome (MDS)/Acute Myeloid Leukaemia (AML) are rare, although the true prevalence is likely to be underestimated. The clinical utility of genetic testing is extensive as it aids prognostication, patient management and identifying at-risk relatives. At least ten genes are linked to familial MDS/AML, involved in transcription regulation, telomere maintenance and splicing. Our laboratory currently offers Sanger sequencing for RUNX1 for familial platelet disorder with propensity to AML, and familial AML with mutated CEBPA. As part of a collaborative LLR funded project with the Barts Cancer Institute to improve testing, diagnosis and management of these patients, we have designed and validated a ten-gene familial MDS/AML NGS panel. The panel was designed using Agilent Sure-Design software and samples enriched using Agilent SureSelect QXT chemistry. Libraries were sequenced using the MiSeq and data analysed using Agilent SureCall software. There was >99% coverage for each gene with at least 100x read depth over two runs for each target region. We have detected known variants in all ten genes, as well as two novel likely pathogenic mutations in the DDX41 gene, a newly associated familial MDS/AML gene. This panel will subsequently be utilised for characterisation of both retrospective and prospective cases from Barts and will be available as a new UK wide and international service for suspected familial MDS/AML patients to increase diagnoses and aid improved patient management.

## P14.038

### Clinical utility of MEFV gene analysis in patients with a clinical suspicion of Familial Mediterranean Fever

**V. Procopio, I. Loddo, A. Romeo, G. Bianco, C. Liuzzo, G. Conti, L. Rigoli, C. Salpietro; UOC Genetics and Pediatric Immunology, University of Messina, Messina, Italy.**

Introduction: Familial Mediterranean Fever (FMF) is an auto-inflammatory disease characterized by brief recurrent episodes of fever and serositis that result in abdominal, chest, joint and muscular pain.

#### Materials and methods

We tested 31 patients and their family members by Reverse Dot Blot analysis.

Results: All patients with FMF showed both homozygous or heterozygous MEFV mutations. Analysis performed on family members allowed us to identify 36 individuals with heterozygous MEFV mutations. Only 53.8% of them presented some clinical manifestations attributable to a mild FMF.

Conclusions: Mutations identified by Reverse Dot Blot analysis covered about 90% of the mutations, as well as that identified by Sanger sequencing. According to the literature, a significant subset of person with FMF (25%) have a heterozygous MEFV pathogenic variant, then the sequencing detection rate of cases not diagnosable by Reverse Dot Blot drops to 67.5%.

The reason may be due to:

- 1) Mutation in another gene;
- 2) Overlapping manifestations with other autoinflammatory diseases that make difficult differential diagnosis.

In literature, rare mutations in other genes have been reported in patients carrying only a heterozygous mutation in MEFV gene.

In heterozygous patients, we consider most useful to perform a six-month trial of colchicine therapy to confirm the diagnosis of FMF and, if no benefit, to analyse other genes responsible for autoinflammatory disease in differential diagnosis with FMF.

We propose a flow chart for genetic testing in FMF, which has an inheritance not well clarified.

## P14.039

### Enabling exome sequencing in non-genetic clinical practice: fast-WES as a routine diagnostic test

**C. A. L. Ruivenkamp<sup>1</sup>, A. C. J. Gijsbers<sup>1</sup>, A. H. B. Bollen<sup>1</sup>, J. K. Vis<sup>1</sup>, I. F. A. C. Fokkema<sup>1</sup>, A. van Haeringen<sup>1</sup>, N. den Hollander<sup>1</sup>, L. Hoefsloot<sup>1,2</sup>, C. van Asperen<sup>1</sup>, M. J. V. Hoffer<sup>1</sup>, G. W. E. Santen<sup>1</sup>**

<sup>1</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>2</sup>Erasmus MC, Rotterdam, Netherlands.

Although whole exome sequencing (WES) is increasingly applied in clinical genetics, it is mostly used to replace the gene-by-gene sequencing paradigm that we were used to. However, several studies have shown that the possible applications of WES stretch beyond clinical genetics: in other clinical situations, particularly in the neonatal intensive care unit (NICU), an unbiased tool to make a diagnosis can be extremely useful.

Several hurdles, such as proven soundness and cost-efficacy, need to be taken before a test can be implemented in a routine clinical setting. To our opinion the long turnaround time for WES has been the major barrier. The full standard WES procedure sometimes takes over six months in diagnostic labs. We managed to reduce the waiting period from several months to one week through developing a protocol that combines Agilent's SureSelectXT workflow with rapid Illumina NextSeq sequencing. The sequencing workflow is seamlessly integrated with our bio-informatics pipeline and in-house analysis software, enabling delivery of WES reports based on high quality exome data within two weeks. We will present our workflow, as well as examples underscoring the utility of fast-WES for physicians and parents in the context of NICU. We believe that fast-WES opens many new possibilities for the introduction of WES in routine clinical practice.

## P14.041

### Improving access to genetic diagnostic tests

**S. Abbs<sup>1</sup>, R. Stojnic<sup>2</sup>, I. Johnson<sup>1</sup>, J. Aleksic<sup>2</sup>**

<sup>1</sup>East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, <sup>2</sup>GeneAdviser, Cambridge, United Kingdom.

The Regional Genetics Laboratory at Cambridge University Hospitals, UK, has partnered with GeneAdviser to develop an online platform to streamline searching, ordering, and paying for clinical exome sequencing and other genetic tests.

Tests can be searched for by gene, by disease, or by test, at [www.geneadviser.com](http://www.geneadviser.com). The methodology, sequencing coverage by gene, cost, reporting time, and the Laboratory's experience with each test are displayed. A "related tests" feature is useful to help clinicians identify additional gene sequencing panels that may be appropriate for patients with heterogeneous phenotypes, searching over 250 gene panels and almost 5000 genes available via our GEMINI clinical exome sequencing. Through an API interface, whenever new tests are added to the Laboratory's database they are almost instantly visible online, ensuring that clinicians have access to tests as soon as they are available.

Electronic ordering of tests through the GeneAdviser secure platform will allow seamless integration with the testing Laboratory's LIMS, improving efficiency in the laboratory and reducing the likelihood of transcription errors. All invoicing and payment for tests are handled centrally through GeneAdviser, meaning that the Laboratory only has to arrange a single periodic invoice and payment for all transactions, avoiding numerous financial transactions and transmissions of supporting documentation with individual clinicians and institutions.

By making it easier for clinicians to find and order genetic diagnostic tests for their patients, and by improving efficiency in the laboratory, this platform will help more patients gain access to the most appropriate and latest tests available.

## P14.042

### GOSHG2P: web-app and database for diagnostic clinical exome analysis

**R. M. Nyanhete, C. Boustred, T. Cullup, L. Jenkins;**

*North East Thames Regional Genetics Laboratory, London, United Kingdom.*

Next-generation sequencing (NGS) technologies have enabled diagnostic genetic laboratories to move from single gene tests to targeted gene panels and clinical exomes. The result has been a rapid increase in the number of genetic variants identified. Legacy approaches to storing and interpreting variants are not scalable and have traditionally used different systems to store variants. To address this problem we have developed a database and web application for variant storage and interpretation.

Great Ormond Street Hospital Genotype to Phenotype (GOSHG2P) is an application that provides clinical scientists with an interface for variant interpretation. All variants identified through our NGS pipeline are stored in a standard format, with annotations of all refseq transcripts consisting of conservation, variation database frequencies and *in silico* tools for pathogenicity prediction. Variant frequencies across sequencing runs are calculated based on virtual gene panels tested. Virtual gene panels can be created and curated within the application. Run quality metrics and a database of PCR primers for variant confirmation are available. GOSHG2P has the capacity to store patient phenotypes, which will aid the interpretation of sequence variants.

GOSHG2P enables clinical scientists to analyse virtual gene panels on our clinical exome that are relevant to the phenotype of a patient or reason for referral without the discovery of incidental findings. Standardisation of va-

riant storage enables variants and their interpretation to be exported using VCF format. This provides a good platform for variant sharing with other clinical laboratories and the wider genetics community for better research into rare disorders.

#### P14.043

#### Comparing Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) assays in two different invasive breast cancer cohorts using 2007 and 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Human Epidermal Growth Factor Receptor 2 (HER2) gene testing guidelines

T. H. Lim, A. S. T. Lim, Y. Y. S. Yeap, J. K. K. Ho, K. C. H. What, S. L. Tien, P. H. Tan; Pathology Department, Singapore General Hospital, Singapore, Singapore.

Introduction: HER2 amplification in breast carcinomas is used as a predictive marker for trastuzumab treatment. IHC and FISH testing algorithms have been based on 2007 ASCO/CAP guidelines with new scoring criteria updated in 2013. This study assessed the impact of the new guidelines on HER2 testing.

Materials and Methods: Retrospective review of 590 invasive carcinomas with concurrent IHC and FISH results based on 2007 ASCO/CAP guidelines was performed from July 2011-June 2013. Ratio of HER2 FISH to centromeric 17 signals of <1.8 was negative, 1.8 to 2.2 equivocal, and >2.2 amplified. Another subset of 486 carcinomas from January 2014-August 2015 was evaluated using the updated guidelines, which defined negative as ratio <2.0 with average <4.0 HER2 copies, ratio <2.0 with ≥4.0 to <6.0 HER2 copies equivocal, ratio ≥2.0 or ratio <2.0 with ≥6.0 HER2 copies amplified. IHC 3+ was revised from uniform intense membrane staining >30% invasive tumour cells to >10%. Concordance rates between the assays were compared in both sets of data.

Results: Overall concordance rate for FISH and IHC was increased from 94.9% to 95.5% with 2013 classifications. Positive IHC concordance increased from 93.1% to 100% but negative IHC concordance declined from 95.8% to 93.8%. Negative FISH cases decreased from 79.2% to 63.8% while FISH positive cases significantly increased from 20.2% to 34.4%. Equivocal cases also increased from 0.68% to 1.85%.

Conclusions: Improvement in overall concordance rate and positive IHC concordance with updated 2013 ASCO/CAP criteria were observed with more patients being classified as HER2 positive.

#### P14.044

#### Comprehensive genetic testing for hereditary cancer with I2HCP: custom design to preserve understanding and control over the whole diagnostics workflow

E. Castellanos<sup>1</sup>, B. Gel<sup>1</sup>, I. Rosas<sup>1</sup>, E. Tornero<sup>2</sup>, S. Santín<sup>1</sup>, L. Sumoy<sup>1</sup>, M. Perucho<sup>1</sup>, I. Blanco<sup>3</sup>, M. Pineda<sup>2</sup>, L. Feliubadaló<sup>2</sup>, J. Brunet<sup>2</sup>, G. Capellá<sup>2</sup>, C. Lázaro<sup>2</sup>, E. Serra<sup>1</sup>;

<sup>1</sup>Institute of Predictive and Personalized Medicine of Cancer (IMPPC), Badalona (Barcelona), Spain, <sup>2</sup>Catalan Institute of Oncology, Barcelona, Spain, <sup>3</sup>Germans Trias i Puig Hospital, Badalona (Barcelona), Spain.

Introduction: We wanted to implement an NGS-based strategy to analyze 122 hereditary cancer genes with diagnostics quality. The challenge was to retain in the diagnostics lab the same understanding and control over all workflow steps reached by pre-NGS strategies.

Materials and Methods: We developed the I2HCP panel, a custom bait library improved by bait behavior monitoring. We customized sample preparation, tested different NGS platforms and created a clinically-driven custom data analysis pipeline.

Results: Training (n=24) and validation (n=40) sets focused on hereditary colorectal cancer, hereditary breast and ovarian cancer and Neurofibromatosis, for which tested gene sets were established by a multidisciplinary team based on clinical utility. Yield and QC parameters reached diagnostics quality and were maintained after the first 150 samples tested in the routine diagnostics. I2HCP strategy achieved an accuracy, analytical sensitivity and specificity greater than 99%, matching Eurogentest recommendations. We provide examples of how the new workflow improved diagnostic sensitivity, solved uncertain clinical diagnoses and allowed the identification of mutations in candidate cancer predisposition genes.

Conclusions: We developed, validated and implemented and NGS-based diagnostics strategy for hereditary cancer preserving understanding and control over the whole process. A global analysis of hereditary cancer genes revealed a complex variation landscape cohabiting with the disease-causing mutation.

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#### P14.045

#### OncovirSeq: Targeted sequencing of viral integration in human tumours

S. Leonard<sup>1</sup>, A. Skowronski<sup>2</sup>, N. Dyer<sup>3</sup>, S. Ott<sup>3</sup>, S. Clokie<sup>2</sup>, C. Woodman<sup>1</sup>, C. Tan<sup>2</sup>, A. Rettino<sup>2</sup>, M. Griffiths<sup>2</sup>;

<sup>1</sup>Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, <sup>2</sup>West Midlands Regional Genetics Laboratories, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom, <sup>3</sup>Warwick Systems Biology Centre, University of Warwick, Coventry, United Kingdom.

High risk human papillomaviruses (HR-HPV) are associated with nearly 100% of cervical cancers, as well as with vulval, anal, penile and oropharyngeal cancers. Integration of HR-HPV into the human genome is an early event following HPV infection *in vivo*, however whether integration of the virus is capable of driving tumorigenesis remains unclear.

To understand the relationship between HR-HPV integration and tumorigenesis, we have developed OncovirSeq, a highly sensitive sequencing approach and analysis pipeline, which specifically identifies viral-human integrant boundaries. We have used Next Generation Sequencing coupled with sonication and a hybridization-based enrichment method.

To date, we have successfully used this methodology to confirm the already known HPV integration sites in the HPV positive cell lines SiHa, HeLa and Caski. Furthermore, using a longitudinal model of replication, we have identified both the early and late integration events which follow transfection of primary keratinocytes with episomal forms of HPV 16 and 18. From this analysis, we have mapped the most common breakpoints in both HPV 16 and 18, as well as those in the human genome. We have also identified those integration sites which may be important in driving clonal expansion. Importantly, we show that some of these HPV integration sites are the same as those found in HPV associated tumours. We are currently developing this technology for its use with HPV positive tumours, as we believe it will be a useful tool for identifying those viral integration sites important for tumour initiation and progression.

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#### P14.046

#### Massively parallel next generation sequencing to investigate the cis-acting genetic modifiers of instability in Huntington disease

A. M. Alshammari<sup>1</sup>, M. Ciosi<sup>1</sup>, S. Cumming<sup>1</sup>, G. Hamilton<sup>2</sup>, K. Nasser<sup>1</sup>, A. Maxwell<sup>1</sup>, D. G. Monckton<sup>1</sup>, The US-Venezuela Collaborative Research Project;

<sup>1</sup>Institute of Molecular, cell and system biology, University of Glasgow, Glasgow, United Kingdom, <sup>2</sup>Glasgow Polymics, Research Institute of Molecular, Cell & Systems Biology, University of Glasgow, Glasgow, United Kingdom.

Introduction: Huntington disease (HD) is an extremely variable inherited neurodegenerative disorder caused by an unstable expansion of a CAG trinucleotide repeat in the huntingtin gene (HTT). The traditional method of diagnosis is limited due to the assumption that the length of the fragment is equal to the repeat number. This may not always be true because there is a variable CCG repeat downstream of the CAGs and also atypical intervening sequences. Moreover, the traditional approaches cannot measure somatic mosaicism of the expanded CAG repeat, or detect variants within or flanking the repeats. We have used Next Generation Sequencing (NGS) to quantify germline and somatic variants in the HD allele.

Materials and Methods: The HD CAG and CCG repeats were amplified from genomic DNA using locus specific primers combined with barcoded Illumina adapters. The PCR products were sequenced using the MiSeq platform and genotyped by alignment of reads against custom reference sequences.

Results and Discussion: We used the MiSeq platform to sequence and genotype HD alleles and the adjacent CCG repeats from more than 490 DNA samples from both HD patients and unaffected individuals. Our data have revealed three atypical allele structures represented in 56 alleles. However, it must be noted that no atypical haplotypes were detected in intermediate or expanded alleles. We observed the degree of somatic mosaicism for patients with HD is correlated with CAG repeat size and age at sampling. NGS is promising diagnostic tool for genotyping HD and potentially replacing existing methods.

## P14.047

### Study of in-silico predictors to assess the pathogenicity of variants in clinical diagnosis

A. del Pozo<sup>1,2</sup>, K. Ibañez<sup>1</sup>, J. Silla-Castro<sup>1</sup>, E. Vallespín<sup>2,2</sup>, P. Lapunzina<sup>4,2</sup>;

<sup>1</sup>Bioinformatics Section. Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain, <sup>2</sup>Centro de Investigaciones Biomedicas en Red de Enfermedades Raras (CIBERER), Madrid, Spain, <sup>3</sup>Structural and Functional Genomics Section. Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain, <sup>4</sup>Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain.

**Introduction:** Next Generation Sequencing (NGS) allows full and simultaneous study of all mutations present in a set of genes. This advantageous feature becomes a difficult issue if it is unable to select those variants that may be causally associated with the phenotype under study.

As first-phase of variant filtering, it is usually carried out the analysis of the deleterious effect of the variants based on phylogenetic conservation, allele frequency and in-silico predictions. However, the extensive use of prediction tools should be accompanied of criteria that allow for general and good practice recommendations to interpret the numerical results in context of clinical suspicion of the patient.

**Results:** In this study we have performed a comparative analysis of a suite of prediction tools aiming to:

a) establish a unified criteria that enables the assignment of cutoffs to each program and their labelling into some predefined functional categories b) based on these values, assess their predictive value using a benchmark set of variants c) finally, discuss the more suitable strategy of use of in-silico prediction programs in terms of the pattern of inheritance of the disease or the haploinsufficiency status of the gene under study.

**Materials and Methods:** It had selected a set of 11 prediction tools and it had obtained prediction values in a benchmark set of more than 20,000 variants (missense, nonsense, splicing, small insertions and deletions) extracted from literature, a filtered version of humpvar from SWISS-PROT and in-house set of mutations.

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## P14.048

### Variants effecting inflammatory expression profiles identified by a novel biological pathway approach

R. Attard, K. Cassar, R. Farrugia, S. Bezzina Wettinger;  
University of Malta, Msida, Malta.

**Introduction:** The genes involved in the generation of an inflammatory immune response are many. Approaches used to date have not yet unveiled the variants responsible for the generation of different inflammatory expression profiles.

**Methods:** In this family-based candidate gene approach, families were selected from the extreme ends of the phenotype distribution of inflammatory expression profiles, as assessed by multiplex ligation-dependent probe amplification assays on whole blood. Exome sequencing of inflammation-related genes was carried out on an Illumina HiSeq4000. SNPs and indels were compared between families with low and high expression profiles. Variants in genes coding for proteins which physically interact, carry out similar/overlapping functions and which participate in the same biological pathway were analysed per family.

**Results:** Patterns of TLR and inflammatory expression profiles tended to be similar in members of the same family. First-degree relatives share a considerable number of variants in inflammation-related genes, while very few differences were observed between families with low and high inflammatory expression profiles. While an accumulation of variants were identified in some TLRs, IRAK and MAPK genes, fewer or no variants were observed in molecules having a more critical role in the TLR signaling pathway including MyD88, and transcription factors.

**Conclusions:** The similar pattern of inflammatory expression profiles in families is possibly due to the presence of inherited factors. Genetic variation influences the fine-tuning of the inflammatory response, where the overall effect on a pathway is an accumulation of the effects of the variants within that pathway.

## P14.049

### A "sequencing artifact" in LEPRE1 causes osteogenesis imperfecta

K. Tveten<sup>1</sup>, Ø. L. Holla<sup>1</sup>, E. Bryne<sup>2</sup>, Ø. L. Busk<sup>1</sup>;

<sup>1</sup>Telemark Hospital Trust, Skien, Norway, <sup>2</sup>Vestfold Hospital Trust, Tønsberg, Norway.

**Introduction:** A child with clinical osteogenesis imperfecta (OI) was referred to genetic testing. Recessive inheritance was presumed. Previous genetic testing of COL1A1 and COL1A2 was negative.

**Materials and Methods**

Exome sequencing was performed and variants were called in all known OI

genes.

**Results:** At first, no obvious causative variants were found. However, a homozygote variant in LEPRE1 caught our attention. It had a high allele frequency in ESP (92.44%), but no entries in 1000G nor ExAC. Because of the big difference in allele frequencies, ESP was contacted. They confirmed the allele frequency, but regarded it as a sequencing artifact. The variant was a frameshift deletion in the last exon of LEPRE1 and had not been detected in our lab previously. The variant passed all quality criteria and was confirmed by Sanger sequencing.

LEPRE1 encodes prolyl 3-hydroxylase 1 (P3H1) which belongs to a family of collagen prolyl hydroxylases required for proper collagen biosynthesis, folding, and assembly. The variant changes the reading frame for the last 18 amino acids and elongates the protein by 10 amino acids. This region harbors a KDEL sequence which retains P3H1 in the endoplasmic reticulum. P3H1 will probably not be able to retain its function when this sequence is altered.

**Conclusion:** This sequence variant is the probable cause of OI in this patient. Caution must be taken when filtering sequence variants where there is big discrepancy between reported allele frequencies.

## P14.050

### A network-based computational approach for the diagnosis and novel gene identification of leukodystrophies and hereditary spastic paraplegias

A. Schlüter<sup>1,2</sup>, M. Ruiz<sup>1,2</sup>, A. Rodríguez-Palmero<sup>3</sup>, J. Martínez<sup>1,2</sup>, C. Redin<sup>4</sup>, J. Mandel<sup>4,5,6</sup>, A. Macaya<sup>7</sup>, A. López de Munain<sup>8,9,10</sup>, C. Casanovas<sup>1,11</sup>, A. Pujo<sup>1,2,12</sup>;

<sup>1</sup>Neurometabolic Diseases Laboratory, Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet del Llobregat, Spain, <sup>2</sup>Center for Biomedical Research on Rare Diseases (CIBERER), ICSi, Spain, <sup>3</sup>Department of Pediatrics, Hospital Universitari Germans Trias i Pujol, Badalona, Spain, <sup>4</sup>Département de Médecine translationnelle et Neurogénétique, IGBMC, CNRS UMR 7104/INSERM U964/Université de Strasbourg, Illkirch, France, <sup>5</sup>Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, <sup>6</sup>Chaire de Génétique Humaine, Collège de France, Illkirch, France, <sup>7</sup>Pediatric Neurology Research Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>8</sup>Hospital Universitario Donostia, Neurology Department, San Sebastian, Spain, <sup>9</sup>University of the Basque Country (UPV-EHU), Department of Neuroscience, San Sebastian, Spain, <sup>10</sup>CIBERNED: Parque científico de Madrid. C/ Faraday 7-28049, Madrid, Spain, <sup>11</sup>Neuromuscular Unit, Neurology Department, Hospital Universitari de Bellvitge, Hospitalet del Llobregat, Spain, <sup>12</sup>Catalan Institution of Research and Advanced Studies (ICREA), Barcelona, Spain.

Leukodystrophies and Hereditary spastic paraplegias (HSPs) are neurodegenerative diseases manifesting frequently with similar clinical pictures, i.e. chronic progressive spasticity, due to common underlying causes and physiopathological mechanisms. Although the genetic basis is partly understood, only a small fraction of cases receives a definitive genetic diagnosis using targeted approaches nowadays. Whole exome sequencing (WES) may improve diagnostic yield and identify novel causative genes. To analyze the derived data, we have developed a global, network-based computational method designed to prioritize disease genes based on the observation that genes causing similar diseases tend to lie close to one another in a network of protein-protein interactions. This information is integrated with a phenotypic disease metric based on Human Phenotype Ontologies (HPO), to score the strength-of-association of proteins with leukodystrophy and HSP phenotypes. Our diagnostic yield from 41 cases analyzed by WES is as follows: i) Positive diagnosis in 17 cases (pathogenic or likely pathogenic variants); ii) Possible diagnosis in seven cases with variants of unknown significance, of which four are non-previously related to HSP or leukodystrophies; iii) Three novel candidate genes; iv) 14 Inconclusive cases. Our analysis links HSP and leukodystrophy to other neurodegenerative disorders and may facilitate gene discovery and mechanistic understanding of white matter and cortical motor neuron diseases.

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## P14.051

### Diagnostic workup, outcome and cost for patients with suspected Lynch syndrome

S. Gebre-Medhin, K. Henriksson, H. Ehrencrona, I. M. Holm;  
Department of clinical genetics, Lund, Sweden.

**Background and aim:** We have performed a retrospective analysis of the family history of cancer, laboratory results and costs for 372 probands with suspected Lynch syndrome (LS), the most common known hereditary CRC condition. The cluster of affected first degree relatives (CFDR) with the largest number of LS-associated cancers was identified in each proband's pe-

degree. Lowest age at cancer diagnosis (LAD) in each CFDR, including also any affected second degree relatives, was determined. Results: 368 patients were investigated with DNA mismatch repair (MMR) functional analyses of which 92 patients (25 %) were considered to have an MMR deficient tumor. Compared to CFDRs with normal MMR function, CFDRs with MMR deficiency had significantly larger numbers of LS tumors as well as lower LAD. A total of 114 patients were subjected to MMR gene mutation screening of which 92 had an MMR deficient tumor, 10 had a MMR proficient tumor, the remaining 12 having unknown MMR function. Among mutation screened patients, 48 (42 %; i.e. 13 % of the entire cohort) had an LS-associated mutation. Mutations were found in MSH2 (46 %), followed by MLH1 (31 %), MSH6 (21 %) and PMS2 (2 %). Total laboratory costs were 248 482 €. Conclusion: Our data indicate that, in selected patients, the application of a multigene panel covering all major hereditary CRC syndromes as a first line investigation would probably be a more rapid and cost effective strategy to identify individuals in benefit of targeted cancer surveillance and treatment.

#### P14.052

#### Characterizing male idiopathic infertile phenotypes based on a panel of 228 genes using Targeted Next Generation Sequencing

R. Cocos<sup>1,2</sup>, F. Raicu<sup>1,3</sup>,

<sup>1</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania, <sup>2</sup>Genome Life Research Center, Bucharest, Romania, <sup>3</sup>Francisc I. Rainer Anthropological Research Institute, Romanian Academy, Bucharest, Romania.

Innumerable combinations of genetic, epigenetic and environmental factors could explain the causes of male infertility. Recent studies have shown that mutations or polymorphisms in new candidate genes are responsible for the majority of idiopathic forms of spermatogenic failure.

Given the recent evolution in the development of Next Generation Sequencing techniques and the growing number of known and candidate genes we designed and developed a targeted sequencing protocol onto Ion Torrent PGM platform that captures the exons of 228 genes AmpliSeq panel to further increase our knowledge on the molecular base of male infertility.

In our pilot study we tested this panel on DNA samples of 15 out of 100 patients with idiopathic infertility that presented no Y chromosome microdeletions. All samples had 97.85% average of target regions with coverage by at least 30 folds. Data were analyzed using a bioinformatics in house pipeline based on GATK, Picard and AnnoVar software for reading alignments, variant calling, filtering and annotation.

Up to date, all analysed patients had a total of 48 possible causative variants in 37 different genes, excluding common polymorphisms, but increasing the number of samples we probably be able to detect more associated variants or mutations.

Our approach provide a robust and sensitive method to detect novel genetic variants and can represent a powerful method for investigating affected male in order to discover new key molecules that could represent in the future the target for personalized drug or gene therapy. This work was supported by the CNCS-UEFISCDI grant PN-II-RU-TE-2014-4-0527.

#### P14.053

#### RNA sequencing analysis of human sperm samples

L. Ramos<sup>1</sup>, S. Santin<sup>1</sup>, G. Rech<sup>1</sup>, L. Bassas<sup>2</sup>, A. Mata<sup>2</sup>, L. Sumoy<sup>1</sup>, S. Larriba<sup>2</sup>,

<sup>1</sup>IMPPC, Badalona, Barcelona, Spain, <sup>2</sup>Fundació Puigvert, Barcelona, Spain, <sup>3</sup>IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

Introduction: Spermatozoa contain, besides the haploid genetic material, functionally viable transcripts that are delivered into the oocyte during fertilization. The sperm gene expression profile could reflect the fertilizing potential of spermatozoa. However, the assessment of human sperm-RNAs, generally characterized by low quantity and high fragmentation, is complex. We aimed to develop a strategy to characterize the human sperm-RNAs by RNA-seq technology.

Materials and Methods: Two different methods were used to isolate spermatozoa from semen (discontinuous density-based centrifugation and swim-up) and three different methods of RNA extraction (Trizol\_Thermo Fisher, NucleoSpin RNA\_Macherey Nagel and miRCURY\_Exiqon) were compared. RNA samples were DNase treated and removed from contaminants with Nucleospin RNA Clean-up XS\_Macherey Nagel. SMARTer Stranded RNA-Seq Kit\_ClonTech was used for library preparation, preceded by a ribosomal RNA (rRNA) depletion step, for which two different procedures (RiboGone\_ClonTech and Ribo-Zero Gold\_Illumina) were compared. Libraries were sequenced in a HiSeq-2500 (2x50 PE).

Results: All sperm isolation and RNA extraction methods provided comparable results in terms of library RNA class representation. However, exploratory analysis pointed to a list of 56 protein-coding genes differentially ex-

pressed between gradient purification and swim-up sperm isolation. Regarding the rRNA depletion step, RiboGone provided better cytoplasmic rRNA removal efficiency than Ribo-Zero Gold (0.48% vs. 13.1% reads mapping to rRNA), resulting in higher percentage of reads mapping to protein-coding genes (75.53% vs. 61.1%).

Conclusions: The application of an optimized low input RNA-seq procedure to sperm-RNA comparative studies may contribute to a better understanding of the molecular mechanisms underlying male infertility.

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#### P14.054

#### The role of massively parallel sequencing in the molecular diagnostics of repeat expansion disorders

J. Radvanszky<sup>1,2</sup>, J. Budis<sup>3</sup>, F. Duris<sup>2</sup>, J. Gazdarica<sup>4</sup>, M. Gabriel<sup>4,2</sup>, L. Kadasi<sup>1,4</sup>, T. Szemes<sup>4,2</sup>,

<sup>1</sup>Institute for Clinical and Translational Research, Biomedical Research Centre, Bratislava, Slovakia, <sup>2</sup>Geneton Ltd, Bratislava, Slovakia, <sup>3</sup>Department of Computer Science, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia, <sup>4</sup>Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

Introduction: In repeat-expansion disorders (REDs) both normal, as well as permutation and mutation range repeat numbers are highly variable, posing a challenge for molecular diagnostics. In the majority of REDs there is still no single method that would allow identification and sizing of the entire allele range. Therefore, at least two complementing methods are generally required, while sequencing has no special place among them. Using myotonic dystrophy (DM) as a model, we developed an STR genotyping algorithm that allows massively parallel sequencing (MPS) to be applied, as a first tier test, for the exclusion of the presence of REDs associated expansions.

Materials and methods: Following a custom HaloPlex based enrichment, sequencing was performed on Ion Torrent PGM. Repeat number estimations from FASTQ data were performed as follows: motif filtering, annotation of the remaining reads with profile Hidden Markov model and final clustering the annotated reads into groups corresponding to individual alleles.

Results: As second generation sequencing (SGS) has standard amplification derived limitations, expanded alleles remained un-amplified and un-detected, making SGS-based testing un-useful for REDs diagnosis confirmation. Assessed genotypes of healthy-range alleles were, however, highly concordant with those generated by conventional methods, suggesting that even SGS allows diagnosis exclusion if two healthy-range alleles are identified.

Conclusions: If reliable STR genotyping/annotation tools are exploited in the sequence variant annotation process, inclusion of disease-associated repeat regions into targeted sequencing panels may, at least partially, extend the possibilities of MPS based diagnostics into the field of microsatellite expansion disorders (financial support: VEGA\_2/0115/15).

#### P14.055

#### Next generation sequencing for the identification of mutations in MDS patients - A comparison between peripheral blood, CD34+ blood cells and bone marrow aspirate

R. Martin, C. Ganster, D. Haase;

University Medical Center Göttingen, Clinics of Haematology and Medical Oncology, Göttingen, Germany.

Myelodysplastic syndromes (MDS) are clonal stem cell diseases of the bone marrow (BM). Acquired somatic mutations account for MDS formation and constitute important markers for diagnosis in up to 90% of MDS cases. Currently BM aspirates are used for molecular genetic analysis of MDS patients. The aim of this study was to investigate if the high sensitivity of next generation sequencing (NGS) allows reliable genetic diagnostics using peripheral blood (PB).

Six patients (3xRCMD, 3xRAEB-2) were included in our pilot study. For all patients genomic DNA was extracted from mononuclear bone marrow (BMMC), peripheral blood (PBMC) and immunomagnetically enriched CD34+ peripheral blood cells (CD34+). Sequencing was performed using the TruSight Myeloid Sequencing-Panel and a MiSeq instrument (Illumina). Reads were aligned with BWA (hg19) and further processed using the software packages Picard, GATK and AnnoVar. Mutations with an allele frequency  $\geq 5\%$  were included in the analysis. T-cells were used to prove somatic origin.

We identified somatic mutations in all patients. Overall we found 13 aberrations in our cohort (3xRUNX1, 3xSRSF1, 2xU2AF1, 1xASXL1, 1xETV6, 1xIKZF1, 1xTET2, 1xTP53). All mutations were detectable in PB (12xCD34+, 11xPBMC). Eight mutations were present in all tested sample types (BMMC, CD34+ and PBMC). Further details including clone sizes and comparisons between different materials will be demonstrated. Our findings indicate that

NGS enables adequate detection of somatic mutations also from PB preparations. We conclude that NGS will improve diagnostics and disease monitoring. Genetic analysis of PB would significantly reduce invasive clinical interventions.

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#### P14.056

##### Gene-based variant classifier to assist medical sequencing

*K. J. van der Velde, E. N. de Boer, C. C. van Diemen, B. Sikkema, K. M. Abbott, L. Franke, R. H. Sijmons, T. J. de Koning, C. Wijmenga, R. Sinke, M. A. Swertz; UMC Groningen, Groningen, Netherlands.*

The widespread application of medical sequencing is seriously hampered by the lack of vast, accurate and fully automated interpretation tools. Existing variant pathogenicity predictors do not include knowledge from the (public) clinical domain such as known genomic variation and its relationship to human health. They cannot reliably perform automated interpretation for clinical DNA/molecular diagnostics. Here, we address this challenge and present a novel method that delivers accurate computational variant classification. We evaluated allele frequencies, putative protein impact and estimated deleteriousness for >100,000 variants from ClinVar and ExAC in >3,000 genes, resulting in gene-specific calibrations of benign versus pathogenic variants. Next, we developed an algorithm that utilizes these data to classify any new variant that may be found within these genes, and ran successful validation on test sets from VariBench and MutationTaster2. The method's power emerged in a clinical setting, where we found an overall yield of 79% (correlation \* coverage) compared to 30-67% of existing tools when applied to 2,359 pre-assessed variants in patients in five different diseases. This high yield is achieved by a large proportion of variants being located in calibrated genes, demonstrating that human clinical expertise implicitly stored in existing interpreted variant lists can be leveraged to accurately and computationally classify new variants in their gene context. This method can also be used in automated whole-genome interpretation. We provide an online service to annotate VCF files, and an executable including source code is downloadable as open source for use in bioinformatic pipelines.

#### P14.057

##### Verification of miRNA expression using nuclease protection and targeted next generation sequencing

*M. M. Reinholtz, Q. Liu, M. Schwartz, P. Roche, B. LaFleur; HTG Molecular Diagnostics, Tucson, AZ, United States.*

Introduction: miRNAs are short, ~22nt RNA sequences that modulate gene transcription and downstream cell behavior. The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) enables users to measure the expression of 2,083 human miRNA transcripts using next generation sequencing. HTG EdgeSeq extraction-free sample preparation chemistry is compatible with FFPE, cell lines, extracted RNA, and plasma.

Materials and Methods: Two studies were conducted to characterize the performance of the HTG EdgeSeq WTA assay in FFPE and plasma sample types. The sample input studies evaluated a range of sample input amounts for both plasma and FFPE sample types. Optimal input range was established based on sample quality (using process controls) and performance (technical correlation and detection of low expression). Reproducibility studies across technical replicates were also performed for both sample types. Results: Read depth, defined as the total aligned counts at the sample level, for FFPE ranged from 524K at the lowest sample input (1.56 mm<sup>2</sup>/well) to 5 million at the highest sample input (12.5 mm<sup>2</sup>/well) with no loss of sample quality or expression sensitivity. Thus, for FFPE samples any input volume over the entire range is acceptable. The recommended sample input for plasma was established as 12.5 µL/well, this volume resulted in a minimum of 642K reads. Pairwise Pearson correlation coefficients on technical replicates ranged from 0.93 to 0.98 for FFPE samples and from 0.82 to 0.98 for plasma.

Conclusions: The HTG miRNA WTA assay allows for highly reproducible expression with low sample input volumes for both plasma and FFPE samples.

#### P14.058

##### Highly sensitive TaqMan®-based microRNA detection for translational research

*L. Wong, C. Liu, F. Hu, T. Gulham, S. Dong; Thermo Fisher Scientific, South San Francisco, CA, United States.*

MicroRNAs (miRNAs) are small non-coding RNA molecules that have been identified as efficacious biomarkers for the classification of tumors and prediction of outcome for many diseases because of their evolutionary con-

servation, unique expression signatures, relative stability, and abundance. Detection of circulating microRNAs in serum, plasma, and other body fluids holds great promise for a non-invasive approach to molecular diagnostic and therapeutics. However, detection of miRNAs in these clinically relevant samples has been difficult, often requiring greater sensitivity. A typical study workflow in translational research involves first identifying differentially expressed miRNAs in normal and diseased sample types by profiling many miRNAs, then screening or validation of a smaller set of relevant miRNAs. We have developed a new method for the detection and quantification of miRNAs that is highly specific and sensitive. The upstream chemistry allows synthesis of miRNA template library that is used in the downstream real-time TaqMan qPCR for miRNA-specific detection. The universality of template synthesis simplifies the workflow and provides the flexibility for scalable content (miRNA coverage). Whether applied to basic or translational research, profiling, screening, or validation, this new and robust method allows detection and quantification of miRNAs to address the unmet needs in workflow and sensitivity that exists today with next generation sequencing and other qPCR technologies, especially with clinical samples. Results show miRNA-specific detection down to a single cell level providing greater confidence for low copy detection to meet the needs of translational research. Data from plasma and serum will be presented.

#### P14.059

##### Suitability of various tissues for mitochondrial genome testing

*S. L. Hume, S. Taylor, L. Vicen, K. Siriwardena, K. Baptista, L. Podemski, P. Lisa, K. Sprysak, M. Somerville; University of Alberta, Edmonton, AB, Canada.*

As next generation sequencing is able to detect low amounts of mitochondrial heteroplasmy, we sought to investigate tissue sources that would be amenable to full mitochondrial genome testing including sequencing and deletion analysis. Our results demonstrate that either first morning urine or buccal swab samples provide high quality DNA suitable for next generation sequencing. We tested multiple samples from a patient and her mother with a complex I deficiency, m.13513G>A (p.Asp393Asn, NADH dehydrogenase). Although this mutation was present at higher levels in urine and buccal samples than in blood, it was detectable by NGS in all specimens. Of note, this mutation was not detectable in either a urine or buccal sample from the mother. In contrast, deletion analysis by Southern blot is generally not possible on urine or buccal samples as the DNA obtained is insufficient. Additionally, long-range PCR testing of the Kearns-Sayre region revealed that urine has low specificity as deletions are frequently detected in normal unaffected control samples. Southern blot analysis using DNA derived from a muscle sample remains the gold standard for mitochondrial testing. We estimate that the sensitivity of Southern blot analysis is 5% for heteroplasmy while the long-range PCR detects below 5%. In our experience, a long-range PCR of the Kearns-Sayre region must be interpreted with other clinical information (such as CN or BN-PAGE or respiratory enzyme studies) and should not be used in isolation. Therefore, our current mitochondrial genome test algorithm involves NGS on urine followed by Southern blot analysis using a muscle sample.

#### P14.060

##### Functional analysis of variants of uncertain clinical significance in components of the TORC1 signaling pathway

*M. Hoogeveen-Westerveld<sup>1</sup>, M. Hosseinzadeh<sup>1</sup>, R. Swenker<sup>1</sup>, R. Ekong<sup>2</sup>, S. Povey<sup>2</sup>, N. Migone<sup>3</sup>, G. Mancini<sup>1</sup>, L. Hoefsloot<sup>1</sup>, F. Verheijen<sup>1</sup>, M. Nellist<sup>1</sup>; <sup>1</sup>Erasmus MC, Rotterdam, Netherlands, <sup>2</sup>University College London, London, United Kingdom, <sup>3</sup>University of Turin, Turin, Italy.*

The mechanistic target of rapamycin (mTOR) complex 1 (TORC1) is an essential protein kinase complex that controls cell growth and metabolism. Mutations in different components of the TORC1 signaling pathway are associated with a broad spectrum of inherited and somatic diseases. We have investigated the effects of >300 variants of uncertain clinical significance (VUS) identified in genes encoding components of the TORC1 signaling pathway, on TORC1 activity. Our work has provided insight into the likely pathogenicity of the tested VUS, the genetic risks in the families segregating the tested variants, genotype-phenotype correlations and structure-function relationships. Investigating the effects of VUS on TORC1 signaling is a useful adjunct to standard genetic testing and can provide essential information for appropriate clinical management. The tests have been validated for use in our diagnostic laboratory. We will present an overview of our work.

**P14.061****Transition to massively parallel sequencing using a multi-disciplinary team approach - the South Australian experience**

*L. H. Rawlings<sup>1</sup>, K. Kassahn<sup>1</sup>, P. Kaub<sup>1</sup>, K. Friend<sup>1</sup>, A. Tirimacco<sup>1</sup>, K. Brion<sup>1</sup>, E. Douglas<sup>1</sup>, L. McIntyre<sup>1</sup>, D. Lawrence<sup>1</sup>, J. Geoghegan<sup>1,2</sup>, H. Scott<sup>1,2</sup>, C. Barnett<sup>3</sup>, J. Liebelt<sup>3</sup>, L. McGregor<sup>3</sup>, E. Thompson<sup>3</sup>, E. Haan<sup>4</sup>, S. Bain<sup>4</sup>, J. M. Fletcher<sup>1</sup>;*

<sup>1</sup>*SA Pathology, Adelaide, South Australia, Australia*, <sup>2</sup>*Centre for Cancer Biology, Adelaide, South Australia, Australia*, <sup>3</sup>*Paediatric and Reproductive Genetics SA Clinical Genetics Service Women's and Children's Hospital, Adelaide, South Australia, Australia*, <sup>4</sup>*Adult Genetics Unit SA Clinical Genetics Service Women's and Children's Hospital, Adelaide, South Australia, Australia*.

Globally, the implementation of Massively Parallel Sequencing (MPS) has expanded genetic diagnoses by delivering exponential amounts of data at reduced labour costs. The Genetics and Molecular Pathology (GMP) Directorate of SA Pathology (South Australia) comprises four laboratories across three tertiary hospitals testing both constitutional and somatic variants for a large number of genetic disorders. Traditionally, genetic diagnoses were siloed to specific laboratories, based on clinical condition. MPS applications are relevant to every laboratory across the directorate, therefore its implementation has required a cooperative and consolidating approach.

Multi-gene panels, whole exomes and genome testing present opportunities to explore causative sequence variants for genetic disorders, once achieved by time-consuming Sanger sequencing. The rapid expansion of complex test requests has required a complementary increase of knowledge in large data management, quality control and complex analyses, and a change in process for accepting requests for testing and reporting.

To facilitate the transition to MPS, a broad, consultative, multi-disciplinary team (MDT) approach was taken, whereby medical and research scientists, bioinformaticians and specialist clinicians contributed to the evaluation and validation of analytical platforms, clinical utility and best reporting practice. Eighteen months post-implementation, this MDT approach continues to sustain the ongoing function and development of the molecular genetic service through regular meetings with three main foci: service development, intake of MPS requests and complex reporting. Clinical-based input and demand is imperative in all.

This presentation will outline key success factors in the transition process from method validation to clinical implementation of MPS testing in South Australia.

**P14.062****Functional analysis of variants of uncertain clinical significance in NF1**

*M. Kroos-de Haan, M. Hoogeveen-Westerveld, R. van Minkelen, Y. van Bever, L. Hoefsloot, F. Verheijen, M. Nellist; Erasmus MC, Rotterdam, Netherlands.*

Inactivating mutations in *NF1* cause neurofibromatosis type 1 (NF1), an autosomal dominant disorder that affects approximately 1 in 3500 individuals. *NF1* encodes neurofibromin, a GTPase activating protein (GAP) for RAS. Loss or inactivation of *NF1* results in increased levels of active RAS-GTP and increased signaling through the RAS-mitogen activated protein kinase (MAPK) pathway. We have performed RAS-GTP pull-down assays to assess the RAS-GAP activity of *NF1* variants of uncertain clinical significance (VUS) identified in our *NF1* patient population. Our preliminary analysis indicates that functional assessment of *NF1* VUS could be a useful adjunct to standard genetic testing to improve molecular diagnostics for individuals suspected of NF1, and could help provide essential information for appropriate clinical management. Functional assessment of *NF1* VUS is currently being validated for use in our diagnostic laboratory. We will present an overview of our work.

**P14.063****Next generation sequencing as second-tier test in newborn screening programs**

*J. Rodríguez<sup>1</sup>, M. Segura-Puimedon<sup>2</sup>, B. Rodríguez-Santiago<sup>1</sup>, M. A. Bueno-Delgado<sup>3</sup>, A. González-Meneses<sup>3</sup>, I. Fernández-Carvajal<sup>4</sup>, M. J. Alonso<sup>4</sup>, Y. González-Irazabal<sup>5</sup>, M. Espada<sup>6</sup>, R. Yahyaoui<sup>7</sup>, L. Armengol<sup>1</sup>, L. A. Pérez-Jurado<sup>8</sup>;*

<sup>1</sup>*R&D, Department Quantitative Genomic Medicine Laboratories (qGenomics), Barcelona, Spain*, <sup>2</sup>*Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain*, <sup>3</sup>*Department of Pediatrics, University Hospital Virgen del Rocío, Sevilla, Spain*, <sup>4</sup>*Laboratorio de Enfermedades genéticas y cribado neonatal, Departamento de Genética Molecular de la Enfermedad, Instituto de Biología y Genética Molecular Universidad de Valladolid-CSIC, Valladolid, Spain*, <sup>5</sup>*Servicio de Bioquímica Clínica, Hospital Universitario Miguel Servet, Zaragoza, Spain*, <sup>6</sup>*Public Health Laboratory Standards, Basque Government Department of Health, Bilbao, Spain*, <sup>7</sup>*Newborn Screening and Clinical Laboratory, Málaga Regional Hospital, Málaga, Spain*, <sup>8</sup>*Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain*.

Introduction: Newborn screening is a publicly-funded health program for

the diagnosis and intervention of genetic disorders that may otherwise have serious clinical consequences. In order to reduce false positives and negatives, shorten diagnosis time and provide genetic counseling, we are evaluating next generation sequencing (NGS) as a second-tier test.

**Methods:** We use dried blood spot samples from positively-identified newborns to prepare NGS libraries. We have developed a panel of 71 genes commonly affected in the disorders detected by these programs. A bioinformatics pipeline identifies rare genetic variants that may be disease causing. Following technical validation, we have retrospectively analyzed 109 samples to establish the sensitivity and specificity and are prospectively analyzing positive hits in real time to assess clinical utility and cost-effectiveness. **Results:** Retrospectively, biallelic mutations were identified in 85.3% of the samples and single allele mutations in 7.3%. Samples in which no mutations were detected (7.3%) corresponded to hypothyroidism in half of the cases. In samples with previous genetic diagnosis (n=33), 100% concordance was found and a second mutation was identified in two samples. In the prospective phase, current turnaround time is 9 days. Among 15 cystic fibrosis (CF) samples analyzed we have found biallelic mutations in 13% cases, single mutations in 53.3% and no mutations in 33.3%. These data are compatible with the high rate of false positives for CF.

**Conclusion:** We have successfully developed an NGS panel that may allow to bypass or redirect confirmatory tests and provide earlier genetic counseling in newborn screening programs.

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**P14.064****Beyond the Human Exome: finding solutions for clinical research NGS-based applications**

*M. Corioni, E. Lin, K. Jeong, A. Vadapalli, F. Useche, M. Visitacion, G. Amparo, M. Ramaiah, C. Pabon, M. Isaksson, D. Roberts; Agilent Technologies, Santa Clara, CA, United States.*

Protein coding genes constitute approximately 1% of the human genome but harbor most of the disease-associated variants. Targeted re-sequencing enables highly sensitive and comprehensive detection of variants and provides insights into the biology behind a given phenotype. With recent major advances in this technology, combined with a better understanding of biological pathways, NGS is now extensively considered for use in clinical research. A comprehensive Exome is a good solution to catalogue variants and provide deep coverage of genomic content from highly curated databases such as CCDS, RefSeq, GENCODE, Vega, MirBase, UCSC known genes, and Human Gene Mutation Database (HGMD®). We describe here several NGS-based solutions to add value to a comprehensive Exome for specific applications. The first is an augmented Exome for clinical research that focuses on improved coverage of disease-related content, especially that relevant to constitutional disease research. We also describe the combination of Exome with OneSeq probes to extend target enrichment applications to the detection of genome-wide copy-number-changes (CNCs), and copy-neutral loss-of-heterozygosity (cnLOH). Finally, we demonstrate a phased Exome coupled to 10x Genomics' linked read technology. The addition of "phasing" probes allows determination of long haplotype blocks. The separation of exonic reads into haplotypes further aids in structural variant and translocation determination, especially for disease causality in complex genotypes. We describe data analysis and performance attributes for each of these platforms, including SNP/INDEL, loss of heterozygosity, copy number variation, phasing, translocation, and resolution of complex compound heterozygote samples.

**P14.065****Using the wrong approach to solve the wrong problem: Sanger validation of single nucleotide variants from next-gen sequencing**

*L. G. Biesecker, T. Beck, J. Mullikin; NIH - NHGRI, Bethesda, MD, United States.*

Many next generation sequencing (NGS) clinical laboratories validate NGS variants using Sanger sequencing, which is costly. To evaluate the utility of this, we performed a large-scale evaluation of Sanger validation of single nucleotide variant (SNV) NGS variants using ClinSeq® data. We used NGS data from 19 genes in five participants, comparing them to Sanger data on the same samples, and found 0/234 discrepancies. We then compared NGS variants in five genes from 684 participants against Sanger data. Of >5,800 NGS-derived SNVs, 19 were not validated by Sanger. Using new primers, Sanger sequencing confirmed 17 of the NGS variants. We conclude that a single round of Sanger sequencing is more likely to incorrectly refute a true positive variant from NGS than to correctly identify a false positive NGS variant. Our validation rate was 99.965%, higher than many medical tests that don't

necessitate orthogonal validation. Validation of NGS-derived variants using Sanger has limited utility. We suggest moving to a model where NGS variants with good quality scores are reported to clinicians, without Sanger validation, and with an estimate of the error rate. Then, clinicians should make a clinical determination as to which patients should undergo post-hoc Sanger validation, based on the testing scenario and the potential downstream clinical utilization of the variant. The overwhelmingly important challenge for our field is the clinical validity and utility of NGS data, not analytic validity. By focusing on analytic validity, we detract from the central issues that need to be overcome to make genomics clinically useful.

#### P14.066

#### Is the \$1,000 genome as near as we think? A cost analysis of next-generation sequencing technologies

K. J. M. van Nimwegen;

Radboud umc, Nijmegen, Netherlands.

**Introduction:** Substantial technological advancements in next-generation sequencing (NGS) have been made, while sequencing costs have rapidly decreased. This contributes to a swift diffusion of NGS technologies into clinical settings. However, since costs of equipment, personnel, and data management, are generally not considered, the real costs of NGS are currently considerably underestimated. This study aims to provide a comprehensive, transparent, and up-to-date overview of the total costs of NGS.

**Methods:** Cost calculations for targeted gene panels (TGP), whole exome sequencing (WES) and whole genome sequencing (WGS) are based on the Illumina NextSeq500, HiSeq4000, and HiSeqX5 platforms, respectively. Five-year lifecycles, 100x (TGP), 70x (WES), and 30x (WGS) coverage, and 15% (TGP), or 75% (WES, WGS) utilization are assumed in the base case calculations. To anticipate future developments, sensitivity analyses are performed in which these factors, as well as capital and operational costs, are varied.

**Results:** Per-sample costs were €1,669 for WGS, €587 for WES and €333 for TGP. Sensitivity analyses showed that very efficient, long-term use of the sequencing equipment, large reductions in both capital and consumable costs, combined with lowering sequencing depth decrease these costs to €1,006 (WGS), €298 (WES) and €205 (TGP).

**Discussion:** Costs for TGP and WES are considerably lower than for WGS. However, this does not imply that these techniques should be preferred in clinical practice, as the choice of NGS approach should be based on a careful trade-off between per-sample costs, sequencing quality, and diagnostic yield. The results of the present study contribute to making such trade-offs.<br>

#### P14.067

#### Next Generation Sequencing, a powerful technique for diagnosis. A large gene experience

M. Roca-Cervera, A. Romera-López, D. Cantalapiedra, D. Diego-Álvarez, L. Pérez-Cabornero, A. Andújar, R. Pérez-De la Fuente, S. Valverde, L. Cano-Bermúdez, M. D. Arcos-Castillo, C. Collado-Micó, Á. Arilla-Codoñer, G. Marco, G. Legarda, D. Valero-Hervás, S. Santillán; Sistemas Genómicos, Paterna, Spain.

Analysis of large genes is one of the main challenges that genetic tests face. NGS technology has helped overcome this situation but in many laboratories, Sanger sequencing still remains the technique of choice for genetic diagnosis. The aim of the present work is to compare both technique's experience analyzing different large genes and to present the diagnostic results of a Marfan syndrome cohort of patients (*FBN1*). We review 492 samples analyzed with our GeneProfile-NGS panels or with Sanger sequencing to evaluate their performances using parameters such as time-consumed, coverage or amount of DNA used. The cases reviewed included analysis of genes *TTN*, *FBN1*, *DMD*, *COL1A1* and *COL1A2* (ranging 51-363 exons). NGS was performed using SureSelect (Agilent) and SOLiD5500 (Life Technologies) or MiSeq (Illumina) platforms. Clinically significant variants identified were confirmed by Sanger. Sanger was performed using BigDye-Terminator-Kit in an ABIPrism3730xl (Applied Biosystems). Genes initially unaffordable to be analyzed by Sanger (*TTN*) can now be sequenced by NGS in a moderate period of time, resulting cost-effective. Gene coverage by NGS and Sanger was similar, around 99-100%. *FBN1* gene was analyzed in 218 Marfan syndrome patients, of whom 23.7% (2-3weeks/patient) were diagnosed by NGS while 27.8% (2-3weeks/patient) by means of Sanger sequencing. However, the time to complete a Sanger sequencing increases proportionally to the number of samples analyzed, while for NGS remains constant. This confirms that NGS is an effective and accurate technology that allows the analysis of large genes in an affordable time.

#### P14.068

#### Evaluation of a custom mendeliome for diagnostic genetic testing

E. Souche, V. Race, A. Corveleyn, L. Dehaspe, J. Vermeesch, J. Van Houdt, G. Matthijs; KULeuven, Leuven, Belgium.

Next Generation Sequencing (NGS) has become routinely used for the diagnosis of various conditions. Although the most comprehensive NGS test for the diagnosis of heterogeneous conditions would be Whole Genome Sequencing (WGS), this test cannot be implemented in a diagnostic setting yet. In order to reduce sequencing costs, laboratories focus on the sequencing of either all genes by Whole Exome Sequencing (WES) or all genes known to be associated to diseases, generally referred to as clinical exome or mendeliome. Several mendeliomes are commercially available but the size of the region they target differs and some genes listed in the Clinical Genomic Database are not included in any test. We thus developed our own mendeliome by selecting the genes offered in commercial panels, those listed in the Clinical Genomic Database as well as a few more recently published genes. In addition, we included targets for 31 SNPs used for sample tracking. The custom mendeliome protocol is based on Nimblegen SeqCap target enrichment technology. It targets the exonic regions of 5,811 genes, representing a target size of 16.5 Mb and a clinical target (coding bases +/- 2 bp) of 12.8 Mb. The performance of our custom mendeliome and analysis pipeline was evaluated by sequencing well characterized cell lines. The results of the target genes were compared to the full genome information from the platinum genomes project. About 96% of the clinical target could be reliably genotyped. The sensitivity and specificity of genotyping single nucleotides was assessed prior to transfer to clinical diagnostics.

#### P14.070

#### niPy - Reproducible and scalable NGS workflows for pathology: Raw data to validated report

D. Brawand<sup>1</sup>, C. Wall<sup>1</sup>, P. Rushton<sup>1</sup>, L. Steedman<sup>1</sup>, M. Oakley<sup>1</sup>, F. Smith<sup>1</sup>, B. Clark<sup>1,2</sup>,

<sup>1</sup>Viapath at King's College Hospital, London, United Kingdom, <sup>2</sup>King's College London, Molecular Haematology, Division of Cancer Studies, London, United Kingdom.

Next-generation sequencing (NGS) has been widely adopted in diagnostics, and is set to replace Sanger sequencing as the Gold Standard for clinical applications. The complexity of the analysis process, breadth and quantity of data and the necessity for a complete audit trail, with a highly reproducible analytical process presents an operational challenge to laboratories wishing to transition to NGS.

Here we present an open source, clinically validated diagnostic framework which integrates four main components of a genetic testing workflow: A) NGS data processing, B) trend monitoring of quality control metrics, C) variant classification, and D) automated variant validation. Reporting is possible via a standard pathology LIMS using database outputs to reduce transcription errors. A simple web frontend with PDF reports and worksheets enable clinical and laboratory staff to analyse patient data, calculating variant observation frequencies and reviewing pathogenicity scores. Filtering of benign sequence variants, confirmation primer design and SNP check are fully automated.

niPy is version controlled and packaged in cloud-ready application containers allowing rapid replication on commodity hardware, computational clusters or cloud platforms with limited revalidation.

We have successfully integrated niPy into our diagnostic workup of rare anaemia cases and have successfully analysed data from 240 cases, with 15 having novel pathogenic variants including deletions >50bp in size. The turnaround time from raw data to validated variants for a batch of 16 samples is less than 4 weeks. niPy is a validated structured framework for analysis, confirmation and reporting of NGS data in a clinical laboratory.

#### P14.071

#### False-negative in Sanger confirmation of a deletion in BRCA2 found by Next Generation Sequencing (NGS)

R. Muguerza, J. S. Crettaz, L. Otaolea, R. Sáez, Y. Ramírez;

Genetics Department, Donostia University Hospital, San Sebastian, Spain.

**Introduction:** NGS techniques have increased the possibilities of genome analysis but there is still some reluctance to reporting results without Sanger sequencing (SS) confirmation. We present a case where SS failed to confirm a deletion in BRCA2 found by NGS.

**Materials and methods:** Amplicon library of coding exonic and flanking intronic regions of BRCA1/BRCA2 was performed and sequenced by NGS (MiSeq, Illumina). Pathogenic, likely pathogenic or unknown variants and regions with depth <100x were confirmed by SS.

**Results:** A heterozygous deletion was found by NGS in BRCA2: NM\_000059.3:c.4000\_4001del (depth=4996x, allele frequency=44.5%).

Amplification with M13-tailed PCR primers usually used in our laboratory and SS showed absence of the deletion, making us suspect a NGS false-positive or a sample swap error. PCR amplification and SS of a new DNA extraction showed in the electropherogram very slight peaks consistent with the deletion. New primers were designed targeting a larger template that included primer-binding sites, and SS confirmed the deletion this time. Searching for possible causes for the false-negative in SS, we found by NGS a variant (c.4068G>A) in the reverse M13-tailed primer binding site. This variant was absent in the first SS but was confirmed with the new primers. The segregation study supported the hypothesis that the variant was in *cis* with the deletion and caused an allele drop-out.

Conclusion: Although SS is the gold standard sequencing method, false-negatives can occur because allele drop-out, which did not occur in NGS, that was able to detect both the deletion and the variant.

#### P14.072

##### Impact of high plasma cell-free DNA linked to hemostasis abnormalities on non-invasive prenatal testing results

**D. S. C. Han<sup>1</sup>, P. Jiang<sup>1</sup>, D. Y. M. Lam<sup>2</sup>, W. B. Lui<sup>2</sup>, R. W. K. Chiu<sup>1</sup>, Y. M. D. Lo<sup>1</sup>;**  
<sup>1</sup>Li Ka Shing Institute of Health Sciences, Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, <sup>2</sup>Xcelom Ltd, Tai Po, New Territories, Hong Kong.

Introduction: Abnormal clinical non-invasive prenatal testing (NIPT) results have been linked to fetal and maternal chromosomal factors such as fetal mosaicism and maternal cancer. Few studies have investigated other biological confounding factors in NIPT analysis. Identifying factors that can affect NIPT analyses can improve future maternal clinical care.

Methods: Total plasma cell-free DNA (cfDNA) concentrations are monitored for samples undergoing NIPT at a Hong Kong testing service. Plasma cfDNA levels were quantified as genomic equivalent per mL by PCR or fluorometry. Samples with total plasma cfDNA concentrations more than +5 SDs from the mean were investigated. Further analyses of plasma cfDNA were conducted via massively parallel sequencing +/- targeted sequencing to obtain fetal fraction and cfDNA size.

Results: Five cases were identified. Four of these five cases had shorter maternal and fetal plasma cfDNA fragments compared with corresponding reference groups. Three of these five cases had fetal DNA fractions lower than the minimum requirement for NIPT service, necessitating a redraw. A scrutiny into clinical history revealed that each case was associated with an abnormality in the hemostasis system.

Conclusions: These cases show that the ability to report NIPT results are affected by high plasma cfDNA, the levels of which may be linked with the hemostasis system. Further research is necessary to elucidate this link. Meanwhile, these cases attest to the importance of including clinical history in NIPT forms and suggest that patients with hemostasis abnormalities should be pre-counselled of their higher chance of non-reportable result.

#### P14.073

##### Benefit of repeated sequencing of old next-generation sequencing libraries with novel sequencing chemistry on IonTorrent PGM

**K. Soltys, E. Nagyova, D. Dvorska, M. Sekelska, G. Minarik;**  
<sup>Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia.</sup>

As next-generation sequencing (NGS) methods are continuously developing novel version of sequencing chemistries come to the market. In case of NGS analysis on IonTorrent PGM recently HiQ version of emulsion PCR and sequencing kits became available. In our study direct comparison of performance of previously used and recently available sequencing chemistries was performed.

NGS libraries prepared with Haloplex method were amplified by emulsion PCR with use of Ion PGM OT2 and Ion PGM HiQ OT2 kits with subsequent sequencing analysis of amplified libraries with Ion PGM sequencing 200 kit v2 and Ion PGM HiQ sequencing kit. Standard bioinformatic analysis on Torrent server was performed for coverage analysis and variant calling. Hundred of SNP and hundred of InDel variants were used in comparison between the kit performance.

The utilization of new and improved emulsion PCR and sequencing kits led to gain of sequencing data with significantly increased sequencing accuracy and more uniform coverage of gene targets. The homopolymer associated errors were completely disappeared in more than third of previously identified positions and gone to nondetectable in another third. From the point of view of coverage uniformity, standard deviation of coverage decreased from 180 to 156.

Reanalysis of archival NGS libraries with new version of library amplification and sequencing could lead to improved results with more accurate vari-

ant calling and higher uniformity of target coverage. Study was supported with grant VEGA-1/0048/14.

#### P14.074

##### Lyso-SM-509 is an easy-measurable and sensitive biomarker for NP-C: a one-year study

**A. Rolfs, C. Cozma, G. Kramp, S. Eichler;**  
<sup>Centogene AG, Rostock, Germany.</sup>

Niemann Pick Type C (NPC) disease is an autosomal recessive disease caused by mutations in NPC1 /NPC2 genes translated in defects of the lysosomes transport system (cholesterol transporters) leading to abnormal accumulation of cholesterol and glycolipids in the lysosome. Although other organs may be affected (e.g. hepatosplenomegaly), NPC is characterized mainly by progressive neurological deterioration. Recently developed treatment renders the NPC diagnosis of high importance.

The levels of lyso-SM-509 in blood reflect the burden of the NP disease and it can be used for the easy diagnosis of NP patients and for monitoring of the disease progression. Determination of lyso-SM-509 is performed by LC/ MRM-MS, an analytical method proven to be reliable and reproducible both in plasma, serum, EDTA blood and dried blood spots (DBS). Moreover, by combining the results with the levels of lyso-SM-465 the type A/B and type C patients can clearly be distinguished.

We identified 152 NPC patients using lyso-SM-509 and the diagnosis was directly confirmed by sequencing of the NPC1 and/or NPC2 gene. Lyso-SM-509 has a sensitivity of 100% and specificity of 96.5% for the diagnosis of NP. We have identified a total of 300 pathological alleles in the different NPC cases. 63 unique variants (54.4% of total found) are described in CentoMD exclusively. Conclusion: All cases identified lyso-SM-509 biomarker were genetically confirmed proving its high specificity. The ease of DBS based lyso-SM-509 makes the marker an ideal parameter for the simple and confident diagnosis of NP as well as the monitoring of the diagnosed NP patients.

#### P14.075

##### Supplemental CNV analysis in NGS genepanel data in a diagnostic setting

**J. J. Saris, R. van Minkelen, M. A. van Slegtenhorst, H. T. Brüggenwirth, L. H. Hoefsloot;**  
<sup>ERASMUS MC, Rotterdam, Netherlands.</sup>

Standard diagnostic laboratory flows based on PCR and Sanger sequencing often include MLPA analysis when available and relevant. Introducing NGS genepanels in a clinical setting promises a higher diagnostic yield, but is usually used only for sequence variant detection. Normalized Depth-of-Coverage (DOC) calculation can indicate exon copy number variation (CNV) and might replace MLPA. Our lab has established a NGS sequencing flow using JSI SeqNext software. Recently we validated the effectiveness and usability of a DOC-CNV submodule using data from genes located on chromosome X and positive controls. Type and number of reference files (1-on-1 and n=12), signal-to-noise ratio and detection cut-off were evaluated in SeqNext and independent via Z-score analysis.

Aim: Retrospective analysis of genepanel experiments (232 samples, half cardiomyopathy), comparison to validation results and prospective analysis of future experiments (±12) for exon-CNV and wetlab confirmation of calls  $\geq 2$  exons and <5% frequency, using an internal database, including a filter of "noisy" ROI.

Results: In 232 samples (19 experiments) DOC based gender determination using chromosome X located genes, calling was concordant with the recorded clinical gender. Six multi-exon (a.o. RFN170 & SLC20A2) and 10 whole gene CNV (a.o. NPHP1, ACTB) were detected, eight confirmed and eight ongoing. Manual curation of single exon CNV indicates deep intronic InDels influencing DOC results on capturing and/or mapping level, e.g. 5' ANKRD1-exon4 (MAF  $\pm 30\%$ ).

Conclusion: CNV detection using DOC-analysis in SeqNext promises to be an possible replacement of MLPA in diagnostics. Validation, update on results and lessons learned will be presented.

#### P14.076

##### NGS-based measurement of gene expression of 2532 oncology-related biomarkers in formalin-fixed, paraffin-embedded (FFPE) tissues

**D. M. Thompson, M. Reinholz, I. Botros, M. Rounseville, P. Roche;**  
<sup>HTG Molecular Diagnostics, Tucson, AZ, United States.</sup>

Introduction: The HTG EdgeSeq Oncology Biomarker Panel Assay (OBP) combines our proprietary quantitative nuclease protection chemistry with

Next Generation Sequencing (NGS) to enable extraction-free detection of 2,568 mRNA transcripts (2,532 oncology-related) from different sample types including formalin-fixed, paraffin embedded (FFPE) tissue. We established linearity and reproducibility assay performance characteristics. Methods: Lung, breast, prostate, and colon carcinomas and melanoma FFPE tissue lysates, THP-1 and HCC78 cell lines, and Universal RNA (URNA) were used for linearity and sample input studies. URNA was used to demonstrate reproducibility of the assay across multiple days and processors. Linear regression R2 and Pearson correlation coefficients (r) were used to assess linearity and reproducibility of the assay.

Results: The R2 for linearity across four concentration points for lung FFPE tissue (6.25-0.78mm<sup>2</sup>), cell lines (1875-234 cells), and URNA (12.5-1.56ng) were >0.97, 0.99, and 0.99, respectively. The r between low (1.56mm<sup>2</sup>) and high (12.5mm<sup>2</sup>) sample inputs for each FFPE tissue type was > 0.98. The r's for intra-run, inter-day, and inter-run reproducibility were >0.95, >0.98, and >0.98. Differential expression of tissue-specific genes was identified in the respective FFPE tissues, including NKX2 and MUC1 in lung, ERBB2 in breast, NKX3, KLK2, and KLK3 in prostate, and SPP1 and PRAME in melanoma.

Conclusions: The HTG EdgeSeq Oncology Biomarker Panel Assay is linear over a wide range of sample inputs, can comprehensively analyze small, clinically relevant tissues, and is highly reproducible. The demonstrated performance of the assay in several FFPE tumor samples enables multiplex oncology biomarker profiling of malignant neoplasms.

#### P14.077

#### PCR based target enrichment for NGS panels and Sanger variant confirmation

*S. Lefever<sup>1,2</sup>, J. Vandesompele<sup>1,2</sup>, F. Coppieters<sup>1,2</sup>,*

<sup>1</sup>Ghent University, Gent, Belgium, <sup>2</sup>pxlence, Dendermonde, Belgium.

Targeted resequencing is an important application in clinical diagnostics. Due to its flexibility in design, high sensitivity and specificity, the polymerase chain reaction (PCR) is ideally suited as enrichment strategy.

Using our in-house primer design tool primerXL, we have generated almost one million PCR assays for both fresh frozen and formalin-fixed paraffin-embedded samples, covering over 98.7% of the human exome. Assays were designed to limit single nucleotide polymorphisms in primer annealing sites and minimize off-target amplification. We have validated 2200 assays from 200 disease causing genes. Wet-lab success-rate was over 96.5 % upon quantitative PCR testing using uniform conditions. Subsequent next-generation sequencing (MiSeq, Illumina) showed equimolar coverage across the different amplicons with almost 90% of the amplicons having a coverage within 5-fold of the mean. Specificity was very high with almost 95% of the assays having <2% of off-target mapping.

Using our assays, NGS gene panels have already been developed for congenital blindness, deafness and various cancer types using different library preparation methods and sequencing instruments. To date, the Center for Medical Genetics in Ghent incorporates our assays to replace Sanger sequencing-based diagnostic tests with NGS (ISO15189 accreditation). In addition, these assays are used for variant validation of various NGS techniques.

Due to the excellent performance the assays, a spin-off called "pxlence" was recently founded ([www.pxlence.com](http://www.pxlence.com)). Its goal is to provide customers easy access to the predesigned assays, enabling them to enrich any exonic region or confirm any variant of interest through either NGS or Sanger sequencing.

#### P14.078

#### Confined placental mosaicism and DNA based prenatal tests aCGH and NIPT: report of two cases

*L. Barranco, E. Lloveras, V. Cirigliano, A. Canellas, M. Costa, C. de la Iglesia, D. Fernandez, M. Herrero, B. Méndez, N. Palau, M. Piqué, D. Yeste, E. Ordóñez, L. Rueda, A. Plaja;*

*Departments de Genètica i Genètica Molecular. LABCO Iberia. Barcelona, Esplugues de Llobregat, Spain.*

Introduction: As new DNA tests such as aCGH on whole uncultured chorionic villus samples (CVS) and cfDNA aneuploidy screening in maternal blood (NIPT) mainly rely on DNA from cytotrophoblastic cells, discrepancies with true fetal constitution due to confined placenta mosaicism should be expected, as observed in short-term cytogenetic cultures. We report two cases involving both assays.

Material and methods: Case 1. aCGH on uncultured CVS was performed because of maternal anxiety using a targeted BAC array (Cytochip Focus, Bluegnome, Illumina) and followed by cytogenetic analysis on long term CVS culture. Follow-up cytogenetic and FISH analysis using LSI D5S23 and D5S721 probes (Vysis/Abbot, USA) were performed on amniotic fluid cells. Case 2. NIPT (NeoBona plus, Labco/Illumina) was performed for ultrasound

abnormalities and result confirmation by aCGH on uncultured amniotic fluid using ISCA 8x60K array (Bluegnome, Illumina).

Results: Case 1. A 5p15.2 deletion was detected by aCGH in uncultured CVS. Both deleted and normal cells were observed by cytogenetic analysis of cultured cells. FISH and cytogenetic analyses on amniotic fluid showed normal results and 5p15.2 deletion was considered a confined placental mosaicism. Case 2. A 1p36 microdeletion was detected by NIPT. aCGH confirmation on uncultured amniotic fluid resulted in 1p36 microduplication, suggesting a possible confined placental mosaicism for the deletion or a low mosaicism of deleted and duplicated cells not detectable by aCGH and NIPT.

Conclusions: aCGH on non-cultured CVS and NIPT share the same biological limitations of direct analysis of CVS thus occasionally failing to reflect the true fetal chromosome constitution.

#### P14.079

#### Cytogenetics for ploidy

*L. Sunde<sup>1,2</sup>, D. Lilballe<sup>1</sup>, I. Niemann<sup>1</sup>, L. Bolund<sup>2,3</sup>, N. B. Scholz<sup>2</sup>, I. Vogel<sup>1,2</sup>,*

<sup>1</sup>Aarhus University Hospital, Aarhus N, Denmark, <sup>2</sup>Aarhus University, Aarhus, Denmark,

<sup>3</sup>Beijing Genomics Institute/HuDa, Shenzhen, China.

Introduction: Until recently, the standard method for screening for gross genetic aberrations was cytogenetic analyses such as karyotyping. Analysis of DNA, such as aneuploidy screening by multiplexed analysis of DNA markers has advantages, like, speed, price and representativeness as more cells are analysed. However, as pooled DNA is analysed, specificity may be hampered. In hydatidiform mole (HM) genetic analyses are performed to discriminate between diploid HMs that impose a high risk of trophoblastic neoplasia and triploid HMs that have a benign course. Diploid HMs exist in various forms: Both genome sets originating in the father (PP), one genome set from each parent (PM), and mosaics (PP/PM).

Materials and methods: In 270 HMs known to be diploid and 154 known to be triploid by karyotyping and/or flow cytometry, DNA markers were analysed.

Results: 259/270 diploid HMs showed markers indicating paternal genome exclusively and 3 showed markers indicating a balanced biparental genome. These would have been classified as having the parental types PP and PM, respectively, without knowing the ploidy.

8/270 diploid HMs and 154/154 triploid HMs showed maternal and paternal markers with indication of a higher fraction of paternal genome, either because two paternal markers were observed in one locus, or because signals from paternal alleles were higher than the signal from the maternal allele. These observations could either be caused by triploidy with the parental type PPM or by mosaicism PP/PM.

Conclusion: In HMs, cytogenetic analyses are superior to DNA analyses for determination of ploidy.

#### P14.081

#### Detection of TMPRSS2/ERG Fusion Transcript Using TaqMan Assays and the QuantStudio 3D Digital PCR System

*A. Rödel<sup>1,2</sup>, M. Laig<sup>3</sup>, A. Schmitz<sup>1</sup>,*

<sup>1</sup>Bayer Pharma AG, Berlin, Germany, <sup>2</sup>Beuth University of Applied Sciences, Berlin, Germany, <sup>3</sup>Thermo Fisher Scientific, South San Francisco, CA, United States.

Current biomedical research aims at personalized cancer therapeutics, for example in prostate cancer. Due to the high prevalence of the TMPRSS2:ERG fusion which occurs in more than 50% of cases, it seems to be a suitable biomarker for monitoring prostate cancer which can be detected in less invasive sample material such as blood and urine. Use of digital PCR is currently being demonstrated to be a highly sensitive method for reproducible and robust measurements without the use of standard curves. Especially the QuantStudio 3D Digital PCR System from Applied Biosystems has a high point-of-care potential due to the closed, compact and easy to handle system. Aim of this project was to establish a system for the detection of TMPRSS2:ERG fusion on this Digital PCR platform, which can be deployed as a future-oriented method. A TaqMan Fusion and a Gene Expression Assay were used to detect mutant and wildtype alleles, and their performance was optimized by changing the temperature-time profile. RNA isolated from fusion positive VCaP and fusion negative LNCaP cell lines were used as reference material. In wet-lab experiments it was possible to detect TMPRSS2:ERG transcripts with a detection limit of 0.05% fusion portion in a correspondingly high wildtype background. Spike-in experiments allowed the confirmation of an easy workflow. This is a first step forward to apply the assay to prostate cancer samples for monitoring disease and to further validate the significance of the TMPRSS2:ERG fusion as a suitable biomarker. For Research Use Only. Not for use in diagnostic procedures.

**P14.082****Achieving an equitable cross border referral service for genetic testing: findings from a quality & patient care perspective**

C. Brady, E. Ryan, S. A. Lynch, A. Butler, D. Barton;

Department of Clinical Genetics, Our Lady's Children's Hospital, Dublin, Ireland.

Introduction: Our Department is pursuing accreditation under ISO 15189:2012. Clause 4.5 states 'the referring laboratory shall be responsible for ensuring that examination results of the referral laboratory are provided to the person making the request'. Approximately 50% of our samples are referred externally for testing. We request a copy of the report with the sample, which we don't always receive. We have been unable to proactively monitor the return of external reports. A risk assessment was performed to identify clinical risks.

Materials and methods: Database query written to identify outstanding reports January 2012-July 2015. List of cases emailed to external laboratories. Results: 1084 reports identified as outstanding; 863 reports had been reported to clinician no copy to us; 120 analyses ongoing. No notification when reporting targets exceeded. Errors in external laboratory led to failure to report in 3 cases & 36 cases were highlighted as potential errors requiring further investigation.

Conclusions: Audit demonstrated inadequacies in our current system and deficiencies in cross border referral service for genetic testing. Improvements made to our systems to monitor the receipt of outstanding reports & improve patient care. Many laboratories have no system in place for follow of external reports. Directive 2011/24/EU on the application of patients' rights in cross-border healthcare emphasises the potential for European Reference Networks (ERNs) "to facilitate improvements in diagnosis". Our data suggest that ERNs will need to tackle the issue of diverse laboratory practices to ensure patient safety, quality & compliance with cross border referral service for genetic testing.

**P14.083****HaloPlex Targeted Resequencing: an interesting approach for rapid molecular diagnosis of RASopathies in Tunisian Patients**N. A. Ghedira<sup>1</sup>, L. Kraoua<sup>2</sup>, A. Lagrade<sup>3</sup>, E. Kerkeni<sup>1,4</sup>, R. Sakka<sup>1,4</sup>, K. Ben Ameur<sup>4,1</sup>, S. Sfar<sup>1</sup>, F. Chioukh<sup>4,1</sup>, S. Olschwang<sup>5</sup>, J. Desvignes<sup>5</sup>, C. Ktaifi<sup>6</sup>, S. Abdelhak<sup>6</sup>, R. Mrad<sup>2</sup>, K. Monastir<sup>4,1</sup>, N. Lévy<sup>6</sup>, A. De Sandre-Giovannoli<sup>5</sup>;

<sup>1</sup>Research Unit 01/UR/08-14, Faculty of medicine of Monastir, University of Monastir, Monastir, Tunisia, <sup>2</sup>Genetic Department, Charles Nicolle Hospital, TunisEl Manar University, Tunis, Tunisia, <sup>3</sup>Aix Marseille University, INSERM, GMGF UMR\_S 910, Marseille, France, <sup>4</sup>Department of intensive care and neonatal medicine, CHU Fattouma Bourguiba, Monastir, Tunisia, <sup>5</sup>Aix Marseille University, INSERM, GMGF UMR\_S 910, Marseille, France, <sup>6</sup>Laboratory of biomedical genomics and oncogenetics- Pasteur Institute, Tunis, Tunisia.

Background: Noonan syndrome and related disorders are autosomal dominant traits resulting from germline mutations in genes evolving the RAS-MAPKinase signaling pathway. Thus, these traits are collectively called the RASopathies. The phenotype of RASopathies associate typical facial dysmorphia, cardiomyopathies, developmental and intellectual delay, skeletal and cutaneous abnormalities and a higher tumorigenesis risk.

Recent advances in genome sequencing have greatly facilitated the genotyping and identification of causal mutations. In this study, we report our personal experience in using Targeted Sequencing for the molecular diagnosis of a cohort of Tunisian patients.

Methods: We performed Haloplex custom target enrichment and NGS to screen 29 genes commonly mutated in RASopathies or in other overlapping diseases in 30 cases of Tunisian patients with clinical suspect of RASopathies. All pathogenic variants were validated by Sanger Sequencing in patients and available parents.

Results: 27 pathogenic or probably pathogenic mutations were found in 70% of analyzed patients (21/30). 14 variations were already reported in literature. Molecular findings were consistent with the phenotype in the majority of cases. Only in 4 cases, identified variations lead us to review the clinical traits and the initial diagnosis of these patients.

Conclusion: The results demonstrate that this approach captures areas of interest with high specificity and uniformity and detects variants with great accuracy and sensitivity. Targeted sequencing will therefore remain the key to determinate the mutational status in RASopathies with a rapid and cost-efficient manner, and thus, can strongly improve genetic counselling and clinical management of patients.

**P14.084****How reference sequences can lead you astray**F. Khawaja<sup>1</sup>, P. Lombard<sup>2</sup>, J. A. Fairley<sup>1</sup>, Z. Deans<sup>1</sup>;

<sup>1</sup>UK NEQAS for Molecular Genetics, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom, <sup>2</sup>Department of Clinical Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

For molecular test results to be interpreted appropriately within a clinical setting, the use of universal mutation nomenclature e.g. HGVS, and associated reference sequences is crucial.

Locus reference genomic sequences (LRGs) provide a stable genomic DNA framework for reporting mutations and are independent of any changes in transcript numbering, therefore enable accurate location of sequence variants.

The UK NEQAS for Molecular Genetics 2015 external quality assessment (EQA) schemes demonstrate the clinical impact of misinterpreted reference sequences. Three examples from the molecular genetics/pathology schemes are discussed, where despite the provision of accurate reference information, laboratories reported genotyping errors due to inappropriate testing. Long QT syndrome scheme participants were required to test for specific gene mutations and were provided LRGs, however, these were misinterpreted and one laboratory tested the incorrect exons for two EQA cases, resulting in critical genotyping errors being reported and the EQA patient being issued with an erroneous result.

In the familial hypercholesterolemia scheme, one laboratory misinterpreted the LRG provided and reported a false negative result due to incomplete testing. They failed to identify a mutation as only bases 1 to 67 for exon one of the *LDLR* gene were sequenced. However, LRG\_274t1 describes exon 1 as a 284 base pair (bp) transcript that includes 187bp untranslated region. Errors were seen not only in inherited diseases, but several laboratories reported incorrect exon locations for hotspot regions of *PIK3CA* in the colorectal and lung cancer schemes also, highlighting the confusion experienced by a significant number of laboratories across different disciplines.

**P14.085****Title: SunScript™ Reverse Transcriptase: a novel high temperature RT engineered from the HIV-1 group O RT**R. Juárez<sup>1</sup>, L. Menéndez-Arias<sup>2</sup>, D. Weber<sup>3</sup>, A. Schneider<sup>3</sup>;

<sup>1</sup>Sygnis Biotech SLU, Madrid, Spain, <sup>2</sup>Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain, <sup>3</sup>SYGNIS Bioscience GmbH & Co KG, Heidelberg, Germany.

SunScript™ is the name of a new product line based on a novel, highly thermostable reverse transcriptase, engineered from HIV-1 group O RT. It allows reaction temperatures up to 85°C and offers improved efficiency and speed of cDNA synthesis. It performs complete reverse transcription of very long mRNA molecules (at least up to 16 kb) and is the best option to transcribe difficult RNA molecules with high GC content or high degree of secondary structures. Available are RNaseH+ and RNaseH- versions of the enzyme. Here we show basic features of the stand-alone enzyme as well as performance in one-step RT-PCR and RT-qPCR (SYBR Green™) mixes.

We find that the one step reactions also allow for high temperature RT reaction steps, and outperform similar one step kits on the market in terms of sensitivity and temperature range.

**P14.086****Leveraging network analytics to infer patient syndrome and identify causal mutations using patient DNA sequence and phenotype data**S. Shah<sup>1</sup>, A. Krämer<sup>1</sup>, K. Boycott<sup>2</sup>, J. Devaney<sup>3</sup>, G. Eley<sup>4</sup>, R. Felciano<sup>1</sup>, S. E. Hofherr<sup>5</sup>, A. Joecker<sup>1</sup>, K. Kernohan<sup>2</sup>, B. W. Meltzer<sup>2</sup>, A. Muthiah<sup>1</sup>, K. Patel<sup>1</sup>, M. B. Sepriash<sup>2</sup>, B. Solomon<sup>4</sup>, J. G. Vockley<sup>4</sup>, D. Richards<sup>1</sup>;

<sup>1</sup>QIAGEN, Redwood City, CA, United States, <sup>2</sup>Children's Hospital of Eastern Ontario (CHEO), Ottawa, ON, Canada, <sup>3</sup>Children's National Medical Center, Washington, DC, United States, <sup>4</sup>Inova Translational Medicine Institute, Falls Church, VA, United States.

A current challenge for identifying genetic variants underlying rare inherited diseases from next generation sequencing data lies in picking out the true disease causing mutation from the list of hundreds and sometimes thousands of deleterious variants. Ingenuity Variant Analysis is a cloud-based application that provides a suite of algorithms and tools to extract valuable insights from such large amount of genetic variation data by leveraging the large-scale causal network derived from the Ingenuity Knowledge Base. The Knowledge Base is a large structured collection of observations in various experimental contexts with over 11 million findings manually curated from the biomedical literature or integrated from third-party databases. Curated findings include mutations, biological interactions, and functional annotations created from individually modeled relationships between proteins, genes, complexes, drugs, and diseases, along with contextual details such as site and type of mutation, cell and/or tissue specific gene expression, mole-

cular interaction, post-translational modifications, details of the experimental design and methods used, and more. To further improve the platform and improve causal variant detection, we also designed preset filter cascades, implementing best practices for rare Mendelian disease genetic data analysis. To assess case solve rate, we analyzed sequence data from 80 patients, afflicted with severe congenital abnormalities, for which the causal variant was previously known. For each sample, we applied the predefined filters, along with the observed clinical signs and symptoms in the patient. By leveraging analytical tools and the Ingenuity Knowledge Base, we achieved over 30x enrichment in the biologically relevant variants.

#### P14.087

#### Expanding the spectrum of SHOX mutations in Idiopathic Short Stature patients through a custom array CGH.

**D. Babu<sup>1</sup>, I. Fusco<sup>1</sup>, A. Fanelli<sup>1</sup>, R. Muniswamy<sup>1</sup>, A. Kozel<sup>1</sup>, S. Mellone<sup>1</sup>, S. Vannelli<sup>2</sup>, M. Giordano<sup>1</sup>;**

<sup>1</sup>University of Eastern Piedmont, Novara, Italy, <sup>2</sup>Ospedale Regina Margherita, Turin, Italy.

Mutations and deletions within the pseudo-autosomal Short Stature Homeobox gene (SHOX, Xp22.33 / Yp11.3) and its enhancer are reported in 2-15% of children diagnosed as Idiopathic Short Stature. Currently molecular diagnosis for SHOX deficiency is carried out by sequencing the coding regions of SHOX and targeted copy number detection assays for the identification of deletions/duplications.

In this study, we screened about 350 ISS patients through sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) assay. Thirty patients (8.5%) were identified with mutations involving SHOX or its enhancers. In order to see whether there were some patients with alterations not detected through these standard procedures we designed a CGH array (Agilent 8x60K) with a coverage of 8000 probes within the PAR1 region (compared to the 26 probes of MLPA) and additional widely spaced backbone probes.

Nineteen ISS patients tested negative with standard methods were analysed with the custom aCGH. Three patients were identified with deletions or duplications in the SHOX area that are likely contribute to ISS. Two deletions of 12.3 kb and 7kb downstream of SHOX, and an amplification including an intron-exon boundary within SHOX were identified. These variations were not reported in the general population. A specifically designed PCR screening on 150 patients identified other 2 patients carrying the 12.3kb deletion. This deletion was not present in 300 controls analysed. This study reveals the importance of custom-designed aCGH in the molecular analysis of ISS that allowed to detect alterations in a further 15% of patients that would have remained undetected.

#### P14.088

#### Stable and predictable TATs: usage of single molecule tags, double tiling, and duplicate smMIP - sequencing

**K. Neveling<sup>1</sup>, B. van Lier<sup>1</sup>, S. Bertens<sup>2</sup>, R. Derkx<sup>1</sup>, E. Bosgoed<sup>1</sup>, A. Rikken<sup>1</sup>, M. Tychon<sup>1</sup>, A. Mensenkamp<sup>1</sup>, D. Zafeiropoulou<sup>1</sup>, S. Castelein<sup>1</sup>, A. Eijkelenboom<sup>3</sup>, A. Hoischen<sup>1</sup>, M. Nelen<sup>1</sup>;**

<sup>1</sup>Department of Human Genetics, Radboud University Medical Centre, Nijmegen, Netherlands, <sup>2</sup>Department of Mechanical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, <sup>3</sup>Department of Pathology, Radboud University Medical Centre, Nijmegen, Netherlands.

**Background:** Molecular Inversion Probes (MIPs) can be used for enrichment preceding sequencing, and have been shown to result in high quality sequencing data. Usage of single molecule (sm) tags improves data quality, by removal of duplicate reads constructing consensus sequences free of experimental artifacts.

**Methods:** We have created a fully automated workflow to perform smMIP-based enrichment. Sequencing is performed using a NextSeq500 (Illumina). Pre- and post-PCR Hamilton robots are trained to perform pre- and post-hybridization pipetting. Automated file handling, data transfer and SeqNext analysis (JSI) delivers high quality sequencing data. Both single genes smMIP setup and gene panel strategies were tested in the optimized workflow. The small insert size of smMIPs allows usage of FFPE-derived DNA.

**Results:** We managed to implement one single protocol for both gDNA and FFPE-derived DNA. By advanced automation, time from DNA isolation to interpretable data is only 4,5 days. Sequencing twice a week is sufficient to handle several hundred samples per week, and sequencing more often only reduces TAT when weekend shifts are included. Single molecules, double tiling and duplicate sequencing deliver very robust data, with Sanger+ sensitivity and specificity.

**Conclusions:** The newly developed workflow is exceptionally stable and predictable in terms of test completeness and outcome. It is functional for gDNA as well as FFPE-derived DNA. To our knowledge this is the first time that

FFPE DNA can be handled using identical automated enrichment protocols. Based on these results, additional panels are currently implemented. Also CNV analysis based on smMIP data is likely feasible.

#### P14.089

#### Minor Variant Finder: a new software for detecting somatic mutations at low level in Sanger sequencing traces

**E. Schreiber, H. Leong, S. Berosik, J. Marks, S. Schneider, W. George, H. Sangha, A. Gerstner;**

*Thermo Fisher Scientific, South San Francisco, CA, United States.*

We have developed software that detects and reports 5% minor variants in Sanger Sequencing traces at 95.3% sensitivity and 99.83% specificity. The software calls variants without prior knowledge of location and affords the advantages of Sanger sequencing, of robustness, low error rate, ease of use, human interpretable visual displays of the data, and low cost per sample and target. The software can confirm somatic variants found by NGS. Noise minimization and peak detection algorithms subtract the baseline noise in a control sample from a test sample, detect candidate minor variants, and confirm them in the complementary strand. To test the new algorithms, synthetic mixtures of minor alleles were prepared by combining DNAs containing mutations in known ratios. 25 different amplicons and nine different genes, including TP53, KRAS, BRAF, EGFR, FLT3, RB1, CDH1, ERBB2, and XYL1 from cell line and FFPE DNA were sequenced on 3500, 3730, and 3130 Applied Biosystems Genetic Analyzers. 632,452 base positions and 2334 total variant positions, spanning variants at 2.5% to 50% were interrogated. The software achieved 95.3% sensitivity and 99.83% specificity for automated detection of 885 variants present at the 5% level in 238,179 high quality base positions. Sensitivity was 98.8% for variants between 7.5-10%, 98.7% for variants between 12.5-25%, and 100% for variants  $\geq 25\%$ . The software displays a noise-minimized trace view to facilitate visual inspection to confirm candidate variants. Further, reference sequences with hg19 chromosomal locations and NGS vcf files can be imported and aligned with the Sanger sequencing data for orthogonal verification.

#### P14.090

#### Low-frequency and rare variants in type 2 diabetes mellitus by exome-sequencing

**I. Galan-Chile, D. Pérez-Gil, V. Martínez-Barquero, P. Cristina, P. Marín-García, A. García-García, F. Chaves;**

*Biomedical Research Institute Hospital Clinic of Valencia- INCLIVA, Valencia, Spain.*

**Introduction:** Type 2 diabetes mellitus (T2DM) is the result from the interaction of environmental, genetic and acquired factors. Low-frequency and rare variants could explain an important fraction of the estimated genetic component of the disease which could be found in the exome. This study has the aim to identify genetic variants in the exome in relation to T2DM in Spanish population.

**Materials and Methods:** Exome sequencing in 200 patients with T2DM and 200 Spanish healthy controls; all subjects had a BMI between 25-34.9 kg/m<sup>2</sup> and were 40 to 65 years old. Exome regions were captured and sequenced by NGS using Illumina systems. A bioinformatic analysis pipeline was used to perform quality controls, to align the reads to a reference genome and identify genetic variants.

**Results:** It was identified 21,822 SNPs in controls and 17,238 in cases with functional effect, present only in controls or cases that meet quality criteria. In particular, 160 and 132 SNPs were splicing variants, 1,817 and 1,614 SNPs were missense variants and 102 SNPs and 50 SNPs were stop variants in controls and cases, respectively.

**Conclusions:** We have identified a large number of genetic variants which may be involved in the development of T2DM or in the protection from it. In order to establish the true genetic variants involved in the disease we will need to validate them by different strategies, replication in a large sample of controls and diabetics as well as carrying out functional studies

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#### P14.091

#### Multiplex PCR and NGS in detection mutations of target genes associated with hearing loss

**V. Mikstiene<sup>1</sup>, I. Vebrate<sup>2</sup>, G. Alzbutas<sup>2</sup>, T. Ranelis<sup>1</sup>, A. Utkus<sup>1</sup>, V. Popendikyte<sup>2</sup>;**

<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Thermo Fisher Scientific Baltics, UAB, Vilnius, Lithuania.

**Background.** The aim of our project was to prepare a versatile pipeline for DNA library preparation suitable for multigene testing. Eleven genes set associated with the sensorineural hearing loss (SLC26A4, MYO15A, OTOF,

CDH23, TMPRSS3, TMC1, TECTA, TRIOBP, TMIE, DFNB59, GJB6) was chosen to assess the clinical utility and feasibility of our method in routine genetic testing.

**Methods.** We employed multiplex PCR and enzymatic fragmentation of amplicons based preparation of DNA libraries followed by the NGS. Forty eight DNA samples of study participants affected with hearing loss were used to test the pipeline.

**Results.** 48 DNA libraries of coding sequences of the 11 genes implicated in development of hereditary hearing loss were constructed. Thermo Scientific™ Phusion™ U Multiplex PCR Master Mix was used to amplify 63 amplicons in one tube. 126 primers were combined into a single reaction for highly specific and efficient DNA samples multiplexing. Sequences of 126 amplicons with a length range from 300 bp up to 5 kb were prepared, fragmentation was performed and NGS data were generated.

We identified five previously published pathogenic SLC26A4 gene point mutations and eight novel missense/splicing variants of SLC26A4, CDH23, MYO15A, TRIOBP genes in our study group, and classified four of them as likely pathogenic.

**Conclusions.** The results of our analysis make this approach promising for wide-scale application. Our pipeline is adjustable for the development of the custom gene panels for targeted gene sequencing applications.

#### P14.092

#### Coverage profile variability, and gene prioritization inferred from comprehensive statistical analysis of targeted NGS diagnostic panels data

I. Roca<sup>1</sup>, S. Gouveia<sup>1</sup>, J. Maynou<sup>2</sup>, M. Couce<sup>1</sup>, L. Palacios<sup>2</sup>, A. Fernández-Marmiesse<sup>1</sup>;

<sup>1</sup>Unit of Diagnosis and Treatment of Congenital Metabolic Diseases. Department of Pediatrics. Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain,

<sup>2</sup>Progenika Biopharma, a Grifols company, Derio, Spain, Santiago de Compostela, Spain.

**Introduction:** Sufficient base-pair coverage is the foremost requirement for the reliable detection of genomic variants using Next Generation Sequencing (NGS) technology, being the uniformity of coverage profiles essential for applications such as copy-number variants (CNVs) detection. Due to the imperfect performance of whole exome captures (WES), genetic laboratories have developed custom gene panels to increase coverage of relevant disease genes.

**Material and methods:** This work comprised the data from 90, 80 and 100 DNA samples analyzed with targeted-NGS panels (in-solution hybridization method and subsequent sequencing in MiSeq platform) for mitochondrial diseases, epilepsy and myopathy respectively.

**Results:** We present a comprehensive coverage and variant pattern analysis from data generated through the routine clinical use of such NGS panels. It shows the influence of GC content, DNA integrity, enrichment kit design, sample processing and mean sample coverage in the coverage profile uniformity and stands for the best conditions to perform a more effective CNV analysis. It also depicts the development of a gene specific score which informs about its specific conservation along evolution and mutational rate, and the subsequent implications on the pathogenicity prioritization of novel mutations found. The higher efficiency of panels with respect to WES and the importance of developing a powerful population variant database are also shown.

**Conclusions:** Detailed knowledge of coverage and variant pattern can be used to achieve a more efficient evaluation of future patient's dataset in order to 1) develop effective algorithms for detection of CNVs and 2) to assess the possible pathogenicity of new variants detected.

#### P14.093

#### Telomeric sequences in cell-free DNA are more abundant in plasma than in serum samples in healthy volunteers

A. Zinkova, A. Svacina, A. Horinek, E. Pazourkova, M. Korabecna;

Institute of Biology and Medical Genetics of the First Faculty of Medicine and General University Hospital, Prague, Czech Republic.

**Introduction:** Telomere lengths in leucocytes and plasma samples are analysed with regard to their correlations with various pathological states. Circulating cell-free DNA (cfDNA) represents a biomarker of growing interest in numerous fields of medicine, especially in prenatal diagnosis and oncology. We studied the variations in relative telomere lengths in plasma and serum in young healthy volunteers with regard to clinical utility of both types of samples.

**Materials and Methods:** We performed quantitative real-time PCR (qPCR) to determine relative telomere lengths (telomere/single copy gene - T/S ratio) in plasma and serum of 26 healthy volunteers aged 20-25 years. The DNA was extracted by QIAamp Circulating Nucleic Acid Kit.

**Results:** We found significant differences in the relative T/S ratios between paired plasma and serum samples. These ratios were significantly higher in plasma samples (Wilcoxon test,  $p < 0.0001$ ) than in serum samples. Each sample was rendered by relative telomere length of a control sample. The total amount of cfDNA measured using qPCR on single copy gene was significantly higher in serum in comparison with plasma ( $p < 0.0001$ ).

**Conclusions:** We detected higher abundance of telomeric sequences in plasma than in serum but higher levels of total cfDNA in serum samples. Our results suggest the existence of different mechanisms involved in cfDNA release and clearance in plasma and serum.

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#### P14.094

#### Transdifferentiation of human fibroblasts to osteoblast-like cells as a novel in vitro model for the study of inherited bone diseases

F. K. Cayami<sup>1,2</sup>, D. Micha<sup>1</sup>, I. E. Kooi<sup>1</sup>, B. Zandieh Doulabi<sup>3</sup>, G. Pals<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, VU medical center, Amsterdam, Netherlands, <sup>2</sup>Center of Biomedical Science, Diponegoro University, Semarang, Indonesia, <sup>3</sup>Department of Oral Cell Biology ACTA, University of Amsterdam and VU-University of Amsterdam, Research Institute MOVE, Amsterdam, Netherlands.

**Background:** Inherited bone diseases such as osteogenesis imperfecta and skeletal dysplasia are responsible for 10% of documented Mendelian disorders. The effect of genetic variants can only be studied in bone-related cells such as osteoblasts but bone biopsies are invasive. On the other hand, dermal fibroblasts are easily obtained. We hereby demonstrate a novel method to efficiently derive osteoblasts from dermal fibroblasts, which can be utilized as an in vitro model for the study of osteoblast-dependent inherited disorders.

**Methods:** We developed a novel transdifferentiation method to differentiate fibroblasts directly to osteoblast-like cells without undergoing the intermediate pluripotent state. After culturing fibroblasts in osteogenic medium supplemented with platelet lysate for 21 days, qPCR analysis of osteoblast-specific marker expression was performed at various time points. Alizarin red staining was done to demonstrate the mineralization capability of transdifferentiated cells. RNA sequencing was performed to characterize gene expression during the transdifferentiation process.

**Results:** Transdifferentiated osteoblast-like cells showed higher expression of the early osteoblast marker, Runx2 compared to fibroblasts on day 7 which decreased thereafter. The expression of other markers such as alkaline phosphatase, osteopontin and osteonectin increased after day 14. Nodule formation by alizarin red staining indicated the mineralization properties of transdifferentiated osteoblast-like cells. RNA sequencing confirmed the trajectory to osteoblasts-like cells by the expression of components in the BMP, WNT and insulin pathways.

**Conclusions:** Human fibroblasts can be directly transdifferentiated to osteoblast-like cells by using a novel platelet lysate-based protocol. This opens numerous possibilities for the study of unclassified variants in inherited bone disorders.

#### P14.095

#### In silico validation of small CNVs from a dense SNP array

N. Cosemans<sup>1,2</sup>, N. Brison<sup>1,2</sup>, P. Claes<sup>3</sup>, I. Noens<sup>2,4</sup>, J. Vermeesch<sup>1</sup>, K. Devriendt<sup>1,2</sup>, H. Peeters<sup>1,2</sup>;

<sup>1</sup>Center for Human Genetics, University Hospital Leuven, KU Leuven, Leuven, Belgium,

<sup>2</sup>Leuven Autism Research (LAuRes), Leuven, Belgium, <sup>3</sup>Medical Image Computing, ESAT/PSI, Department of Electrical Engineering, KU Leuven, Medical Imaging Research Center, iMinds, Medical IT Department, UZ Leuven, Leuven, Belgium, <sup>4</sup>Parenting and Special Education Research Unit, University of Leuven, Leuven, Belgium.

**Introduction:** Arrays based on single nucleotide polymorphisms (SNPs) have been successful for the large-scale discovery of copy number variants (CNVs). However, current CNV calling algorithms have a high false positive rate for small CNVs and experimental validation using qPCR or custom arrays is labor intensive and expensive. Therefore, this study looks at the possibilities of an *in silico* validation approach to distinguish between true and false positive CNV calls smaller than 100 kb resulting from existing CNV calling algorithms.

**Materials and Methods:** The study sample contains 646 individuals (159 fathers, 157 mothers and 330 children) from 159 families with non-syndromic autism spectrum disorders. All family members were genotyped using the Illumina Human Omni2.5-8v1 SNP array and CNV calls were made using CNV-WebStore. The resulting 42,464 CNVs (1-100 kb) were validated *in silico* by comparing the intensities of the underlying SNPs to the distribution of those intensities over all individuals.

**Results:** Almost half of the CNVs was classified as random noise based on the underlying SNP intensities. Moreover, comparing the position of parents and children in the distribution revealed false negative calls in parents resulting in *de novo* CNVs that were actually inherited.

**Conclusions:** The proposed *in silico* validation approach allows rapid detection of many false positive CNV calls smaller than 100 kb, which could thus be excluded from labor intensive and expensive experimental validation approaches. Ongoing research will validate included and excluded CNVs by qPCR to determine the sensitivity and specificity of the method.

## P14.096

### Checking the experts: compliance with author instructions regarding HGVS nomenclature and variant submission to databases in genetics and genomics journals

*J. Osmani, P. Taschner;*

*Genrade Center of Expertise Genomics, Leiden, Netherlands.*

**Introduction:** Several genetics and genomics journals list requirements for use of HGVS nomenclature and/or submission of variants and phenotype information to public databases in their author instructions. The rationale is to improve the quality of variant descriptions in manuscripts and access to variant information in databases. We have investigated the January 2016 issue of several journals to determine how effective this is.

**Materials and Methods:** We used the list of genetics and genomics journals created by the Human Variome Project (HVP) (See <http://www.humanvariomeproject.org/resources/genetics-and-genomics-journals.html>). A group of students has checked the publications first for the basic requirements: mentions of the reference sequence used to describe variants and the presence of the variants in public databases. The next step was to check variant and phenotype descriptions with specific attention for predicted protein effects.

**Results:** Authors include statements suggesting variants have been submitted to databases, but the variants were not found or phenotypic information was missing. Often, predicted protein effects in publications cannot be verified in case of altered splice sites without supporting RNA-level evidence or in case of insertions of unspecified nucleotides. Lack of supporting evidence complicates the assessment of disease-causing effects for diagnostic use.

**Conclusions:** In multiple cases, information missing in the publication was specified in public databases, indicating that submission of variants to databases prior to manuscript acceptance might improve the quality of publications. Reviewers and journal editors could help improving manuscript quality by enforcing the existing guidelines and insisting on compliance by authors prior to acceptance of manuscripts.

## P14.097

### Robustness of next generation sequencing-based preimplantation genetic screening (PGS) of chromosome aneuploidies from various cell types with a very low amount of template DNA

*M. Libik, J. Chrudimská, L. Dvorakova, T. Slavik, J. Krajickova, K. Faldik, J. Drabova, H. Zunova, M. Macek jr, M. Macek sr;*

*University Hospital Motol, Prague, Czech Republic.*

**Introduction:** Reliable chromosome aneuploidy detection in a single cell or from very limited amounts of template DNA is a prerequisite for preimplantation genetic screening (PGS) of chromosome aneuploidies. The aim of the study was to assess the minimum amount of DNA in terms of diagnostic robustness and time to diagnosis in next generation sequencing-based (NGS) PGS.

**Materials and Methods:** Single oocytes, polar bodies, blastomeres, trophoblastoderm, somatic ovarian cells, cultured choriocytotes / amniocytes and isolated DNA were used to assess the diagnostic reliability of whole genome amplification (WGA; SurePlex), followed by the VeriSeq NGS assays (Illumina; USA). Single sperm were examined using a modified WGA protocol (PMID: 23565289).

**Results:** All samples with known karyotype were accurately replicated by our diagnostic approach. Novel findings, previous undetected, were confirmed by MLPA and/or array CGH. During the course of the study we detected a broad variety of aneuploidies, one chaotic embryo and one error in second meiotic division. The ability to detect mosaicism was assessed by artificially mixing DNA derived from blood or cultured choriocytotes / amniocytes with known chromosomal constitution. We are able to detect mosaicism in aberrant cells down to 40%, while lower mosaicism could be due to WGA/NGS artifacts.

**Conclusions:** The utilised NGS-based assay is robust and reliable for routine detection of aneuploidies in clinical PGS in DNA derived from various single cell types and is able to unambiguously detect eventual mosaicism.

## P14.098

### Whole Exome NGS at the Geneva Genome Clinic: Example of successful translation to diagnostics through a multidisciplinary team

*E. B. Hammar<sup>1</sup>, S. Fokstuen<sup>1</sup>, P. Makrythanasis<sup>1,2</sup>, M. Guipponi<sup>1,2</sup>, E. Ranza<sup>1</sup>, K. Varvagiannis<sup>1</sup>, F. A. Santoni<sup>2</sup>, A. Mauron<sup>3</sup>, S. A. Hurst<sup>3</sup>, C. Moret<sup>3</sup>, C. Gehrig<sup>1</sup>, J. Bevillard<sup>1</sup>, A. Vannier<sup>1</sup>, S. Gimelli<sup>1</sup>, E. Stathaki<sup>1</sup>, A. Bottani<sup>1</sup>, A. Paoloni-Giacobino<sup>1</sup>, M. Lidgren<sup>1</sup>, F. Sloan-Béna<sup>1</sup>, L. D'Amato Sizonenko<sup>1</sup>, T. Nouspikel<sup>1</sup>, J. Blouin<sup>1</sup>, S. E. Antonarakis<sup>1,2,4</sup>;*

<sup>1</sup>*University Hospital of Geneva, Geneva, Switzerland, <sup>2</sup>University Medical Center, Geneva, Switzerland, <sup>3</sup>Institute of Ethics, History and Humanity IEHZ, Geneva, Switzerland,*

<sup>4</sup>*Institute of Genetics and Genomics of Geneva (IGE3), Geneva, Switzerland.*

The advances of next generation sequencing (NGS) technologies enable their application in clinical care. However, there are several significant challenges for their implementation in clinical diagnostics.

In Geneva, we use whole exome sequencing (WES) followed by targeted bioinformatics analysis of individual gene panels for the diagnosis of Mendelian disorders and we have created a multidisciplinary working group, the Genome Clinic Task Force which meets once a week. During these meetings, clinical cases and results are presented, the class of variant pathogenicity is debated and the final laboratory reports are critically discussed. Reimbursement and ethical issues such as informed consent, disclosure of incidental findings and/or variants of unknown clinical significance (VUS) are also addressed. In Switzerland, reimbursement of diagnostic NGS tests is integrated in the public health insurance since January 2015.

During the pilot year 2015, a total of 144 cases (41 with developmental delay (DD) and 103 with various other mendelian diseases (VDM)) were analyzed. We found pathogenic variants (class 4 or 5) in 27% of patients with DD and in 41% with VDM; the average detection rate of (likely) causative variants was 37 %. The decision to report VUS was made on an individual case basis.

In order to render our diagnostic use of NGS even more efficient and patient-friendly, our current aims are to 1) improve different steps of the workflow in order to accelerate the analysis process 2) continuously update the variant interpretation methods 3) continuously learn from the challenges we encounter during the genetic counseling sessions.

## P14.099

### Dual genetic diagnosis as a valuable feature of whole exome sequencing

*A. M. Bertoli Avella<sup>1</sup>, O. Brandau<sup>1</sup>, K. Kandaswamy<sup>1</sup>, N. Nahavandi<sup>1</sup>, D. Trujillano<sup>1</sup>, P. Bauer<sup>1</sup>, R. Abou Jamra<sup>2</sup>, A. Rolfs<sup>1,3</sup>;*

<sup>1</sup>*CENTOGENE GA, Rostock, Germany, <sup>2</sup>Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Germany, <sup>3</sup>Albrecht-Kossel-Institute, University of Rostock, Rostock, Germany.*

**Introduction:** When establishing a molecular diagnosis, we usually search for a single genetic variant which clarify the full phenotypic spectrum of the patient. However, many patients present with complex clinical features not allowing the clinician to make a clinical diagnosis. In these cases with unusual and blended clinical presentations whole exome sequencing (WES) is especially useful.

**Results:** Here we report 21 families for whom whole exome sequencing provided a clear dual genetic diagnosis. These cases presented with a broader or atypical phenotype that could not be explained by the finding of one unique variant.

In 5 of these families, the diagnoses were based on pathogenic/likely pathogenic variant for both disorders. The rest received a molecular diagnosis based on a pathogenic/likely pathogenic variant, and a second probable diagnosis based on a variant of unknown significance, with significant supportive evidence. These diagnoses are likely due to high compatibility of the gene-related disease with the clinical details and the mode of inheritance provided by the clinicians. Some relevant examples of dual diagnoses were spastic tetraplegia and intellectual disability (*SLC1A4* and *HNRNPU*) as well as Donohue syndrome and immunodeficiency type 28 (*INSR* and *IFNGR2*). Other cases included: cerebellar ataxia - deafness, distal hereditary motor neuropathy type VI - atypical Krabbe disease and primary microcephaly type 3 - Leber congenital amaurosis.

**Conclusion:** WES allows complete and dual diagnostic analysis and a better dissection of gene specific phenotypic characteristics positioning WES as a first line diagnostic tool.

## P14.100

### Clinical sequencing: WGS is the better WES

*J. Meienberg<sup>1</sup>, R. Bruggmann<sup>2</sup>, K. Oexle<sup>1</sup>, G. Matyas<sup>1</sup>;*

<sup>1</sup>*Center for Cardiovascular Genetics and Gene Diagnostics, Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland, <sup>2</sup>Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Berne, Berne, Switzerland.*

**Introduction:** Current clinical next-generation sequencing makes use of gene panels and exome analysis, both of which involve selective capturing of target regions. However, capturing has limitations in sufficiently covering coding exons, especially GC-rich regions. **Materials and Methods:** We compared whole exome sequencing (WES, SureSelect Human All Exon v5+UTR) with the most recent PCR-free whole genome sequencing (WGS, Illumina TruSeq) for five female samples. We assessed the proportion of completely and sufficiently covered ( $>13\times$ ) RefSeq coding exons. Thereby we analyzed (i) the entire exome and subsets of clinically relevant exons, i.e. (ii) genes recommended by the American College of Medical Genetics or (iii) exons with mutations recorded in the Human Gene Mutation Database.

**Results:** PCR-free WGS appears to be insensitive to GC content and is thus able to provide hitherto unprecedented complete coverage of the coding region of the genome. Although the average read depth was less than half ( $65\times$  in WGS vs.  $154\times$  in WES), the proportion of completely and sufficiently covered coding exons was significantly higher in PCR-free WGS for all analyzed set of exons.

**Conclusions:** The advantage of WGS does not only include the potential of identifying non-coding pathogenic variation but, in view of its more homogenous and complete exomic coverage, WGS is the better WES, thereby outweighing the higher costs. Thus, from a clinical/technical point of view capturing is no longer necessary for the most comprehensive genomic testing of Mendelian disorders.

## P14.101

### Whole genome amplification effect on segmental copy-number changes and copy-number neutral loss of heterozygosity analysis by oligonucleotide-based array comparative genomic hybridization

*A. Mikulasova<sup>1,2,3</sup>, J. Smetana<sup>1,3</sup>, M. Wayhelova<sup>3</sup>, H. Janyskova<sup>1</sup>, R. Hajek<sup>4,5</sup>, P. Kuglik<sup>1,2,3</sup>,*

<sup>1</sup>*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, <sup>2</sup>Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, <sup>3</sup>Department of Medical Genetics, University Hospital Brno, Brno, Czech Republic, <sup>4</sup>Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic, <sup>5</sup>Department of Haematology, University Hospital Ostrava, Ostrava, Czech Republic.*

**Introduction:** Whole genome amplification (WGA) is an approach designed to overcome small amounts of DNA for genome-wide genetic tests. Various strategies of WGA have been developed; however, none of them can guarantee the absence of amplification bias.

**Materials and methods:** A total of 4 multiple displacement amplification (MDA)-based and 2 PCR-based WGA kits were compared in their effect on segmental copy-number (CN) changes and copy-number neutral loss of heterozygosity (cnLOH) detection by 3 microarray platforms: CGH/4 $\times$ 44K (Agilent), CGH+SNP/4 $\times$ 180K (Agilent) and CGH+SNP/4 $\times$ 180K (OGT). Genomic imbalances-rich cell line U266 was used as material.

**Results:** The main outcomes are as follows: 1) MDA-based WGs showed higher tendency to generate false positive imbalances in contrast to PCR-based WGs with higher risk of false negativity; 2) the specific risk of false positivity and/or negativity increased with decreasing CN segments size; 3) single-cell WGs showed significantly worse effect on results in comparison to WGs with nanogram level of DNA as input; 4) PCR-based WGs were incompatible with cnLOH analysis based on SNP in restriction digestion sites and also showed higher risk of cnLOH false negativity when combined with analysis based on simple hybridization. Detailed data of each point are presented.

**Conclusions:** The results of this study help to choose WGA according to individual user requirements and options. Moreover, we have shown a strategy to verify and validate segmental CN changes detection by DNA array protocol including any WGA for any purpose to attain the highest efficiency without an unnecessary WGA bias. Supported by MHCZ-DRO (FNBr-65269705).

## P14.102

### Whole-genome sequencing based strategy for diagnostics of rare inherited diseases - a collaboration between the academia and the Swedish healthcare

*V. Wirta<sup>1,2,3</sup>,*

<sup>1</sup>*Karolinska Institutet, Solna, Sweden, <sup>2</sup>Science for Life Laboratory, Stockholm, Sweden,*

<sup>3</sup>*KTH Royal Institute of Technology, Stockholm, Sweden.*

Clinical Genomics at Science for Life Laboratory is a research infrastructure providing access to clinical-grade (ISO 17025) sequencing services. Our vision is to support precision medicine initiatives in the Swedish healthcare by providing a cutting edge infrastructure for clinical-grade NGS work, and through multi-year collaborative projects to prospectively demonstrate the utility of NGS-based tests. The infrastructure includes automated strategies for preparation of samples for sequencing on HiSeq X, HiSeq 2500 or MiSeq

systems. Data analysis is carried out using primarily in-house developed software solutions.

Focus has been on establishing diagnostic strategies for rare inherited diseases. Briefly, exome or whole-genome sequencing is followed by identification and annotation of variants, followed by ranking based on expected pathogenicity. Variants associated with a pre-defined list of genes relevant for the specific disorder are reported.

During the last 24 months we have processed  $>1100$  samples (700 cases) with suspected inborn errors of metabolism, skeletal dysplasia, primary immunodeficiency, unknown syndromes, and neuromuscular disorders. During the last six months a transition to whole-genome sequencing has been carried out and currently 40 to 100 samples are analysed monthly. The median turnaround time was 15 days in 2015. The impact has been dramatic with life-changing therapies initiated and several novel disease genes being identified. This collaboration has allowed clinical labs both with and without in-depth NGS and bioinformatic knowhow to implement WGS-based testing in routine settings.

We present a detailed description of the setup and the successful collaboration between the academia and the healthcare system.

## P14.103

### Is FISH and conventional chromosome analysis essential in genomic array era?

*N. V. Shilova, I. V. Kanivets, Y. O. Kozlova, Z. G. Markova, E. V. Musatova;*

*Federal State Budgetary Institution, Moscow, Russian Federation.*

**Introduction:** Whole-genome chromosomal microarray is recommended as a first-tier clinical diagnostic test in cases of intellectual disability and/or multiple congenital anomalies. In the era of genomic arrays, a role for conventional karyotyping and FISH is widely discussed.

**Results:** High-resolution genome-wide array analysis using Affymetrix Cytoscan™ HD array platform was applied in 28 probands with abnormal phenotype. In each case genome imbalance was detected. FISH for every microarray results was performed not to confirm rather a visualization of the abnormal chromosomes. Double segmental imbalances was detected in 17 cases (61%) and in 15 of them a typical microarray pattern for an unbalanced translocation was shown. Prenatal carriers of reciprocal translocations were estimated by targeted FISH or conventional karyotyping. In two cases a gain and loss were located on the same chromosome and non-recurrent inv dup del (10q) and (5p) have been identified by targeted FISH and mBAND. Single segmental imbalances (or loss either gain) was detected in 11 cases (39%) and confirmed by targeted FISH in 9 cases. In one case a 8.7-Mb loss in 15q11.2q13.3 was revealed to be unbalanced translocation due to adjacent-2 malsegregation of paternal translocation (13;15) (q11.1;q13.3). In case, although microarray testing revealed a loss of entire chromosome , FISH combined with chromosome analysis diagnosed mosaicism 45,X/46,X,psu idic(X)(p11.2)/46,X,r(X)(p11.2q13).

**Conclusions:** FISH evaluation revealed more complex rearrangements than suspected based on the array results. The value of genome imbalance structure by FISH and conventional karyotyping is essential for identifying the type and origin of chromosomal rearrangement, providing accurate genetic counseling.

## P14.104

### Standardizing the quality control of any DNA isolation to safeguard the success of genetic testing

*T. Martens, T. Boonefaes, E. De Raeymaecker, T. Montaye;*

*Trinean NV, Gentbrugge, Belgium.*

Genetic tests become ever more powerful, yet also more complex and costly, which is why it is prudent to include quality control steps throughout the preparation process to ensure the quality of the downstream data obtained. To make an informed go/no go decision and avoid analytical failures, a good assessment of the yield and purity of DNA/RNA isolations is essential, including the detection of carry-over constituents that may interfere with downstream tests. Here, we validate a novel approach for DNA quality assessment using a large DNA sample set derived from a variety of human tissues combined with a wide scale of extraction methods. This new QC tool employs the micro-volume spectroscopy on the Xpose™ 'Touch & Go' reader by Trinean combined with its spectral content profiling software to specifically quantify the isolated DNA as well as the amount of contaminating constituents in the sample contributing to the measured UV/Vis spectra.

## P15 Personalized/Predictive Medicine and Pharmacogenomics

### P15.01

#### Polymorphism of immune response genes in children with infectious complications during antileukemic therapy

M. Avdonina<sup>1</sup>, I. Abramov<sup>1</sup>, A. Ikonnikova<sup>1</sup>, Y. Ammour<sup>2</sup>, T. Nasedkina<sup>1</sup>;

<sup>1</sup>Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation, <sup>2</sup>Federal Research Clinical Center of Pediatric Hematology, Oncology and Immunology named after Dmitrii Rogachev, Moscow, Russian Federation.

**Introduction:** Intensive polychemotherapy during treatment of acute leukemia is associated with pronounced immunosuppression, which is a risk factor for serious infectious complications that significantly worsen the prognosis and clinical outcome. Genetic variability of immune response genes may contribute significantly to the development of different infectious diseases and sepsis.

**Materials and Methods:** To search genetic markers defining susceptibility to infections 24 children with acute leukemia who had heavy infectious complications in the course of therapy were selected. Patient's DNA samples were prepared using Roche NimbleGen Sequence Capture of 17 candidate genes NOD2, NLRP3, STAT1, STAT3, CARD9, IL10, IL17RA, IL12RB1, IL1A, TLR1, TLR2, TLR3, TLR4, PTPN22, IL7R, IL7, MYD88. Pyrosequencing was performed on a Roche 454 GS Junior benchtop high-throughput sequencing platform.

**Results:** The analysis of next-generation sequencing data revealed 39 non-synonymous SNPs leading to amino acid substitutions, including informative genetic markers in the following genes: PTPN22 c.1858C>T (rs2476601), TLR1 c.1805G>T (rs5743618) and c.743A>G (rs4833095), TLR3 c.1234C>T (rs3775291), TLR4 c.896 A>G (rs4986790) and c.1196 C>T (rs4986791), IL7R c.197 T>C (rs1494555) and c.412G>A (rs1494558). Sanger sequencing was used to validate the results of next-generation sequencing.

**Conclusions:** Identification of genetic markers defining susceptibility to infectious diseases will allow estimating individual genetic risk to acquire heavy infections during treatment of acute leukemia in children in order to develop new approaches to accompanying therapy. The work is supported by Federal Target Program of Ministry of Education and Science of Russia (grant №14.604.21.0117, RFMEFI60414X0117).

### P15.02

#### Genome-wide association study on treatment response to anti-VEGF therapy for age-related macular degeneration

L. Lorés-Motta<sup>1</sup>, M. Riaz<sup>2</sup>, M. Grunin<sup>3</sup>, J. Coroninas<sup>4</sup>, F. van Asten<sup>1</sup>, M. Pauper<sup>4</sup>, I. M. Heid<sup>5</sup>, E. K. de Jong<sup>1</sup>, C. B. Hoyng<sup>1</sup>, R. H. Guymer<sup>2</sup>, P. Mitchell<sup>6</sup>, S. Fauser<sup>7</sup>, A. I. den Hollander<sup>1,4</sup>, P. Baird<sup>8</sup>, I. Chowers<sup>9</sup>;

<sup>1</sup>Department of Ophthalmology, Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Ophthalmology, Department of Surgery, University of Melbourne, Melbourne, Australia, <sup>3</sup>Department of Ophthalmology, Hadassah Hebrew University Medical Center, Jerusalem, Israel, <sup>4</sup>Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands, <sup>5</sup>Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany, <sup>6</sup>Centre for Vision Research, Department of Ophthalmology and Westmead Millennium Institute for Medical Research, University of Sydney, Sydney, Australia, <sup>7</sup>Department of Ophthalmology, University Hospital of Cologne, Cologne, Germany.

**Introduction:** Age-related macular degeneration (AMD) is the most common cause of blindness in the industrialized world. Approximately 90% of the visual loss is attributed to the neovascular form of the disease (nvAMD). NvAMD can be treated with intravitreal injections of anti-VEGF agents. While this treatment revolutionized the prognosis of the disease, a remarkable variability in the response has been described. Candidate gene variant analyses have been inconsistent in explaining this variability, therefore, hypothesis-free approaches are needed in order to assess the role of genetic variation in treatment response.

**Methods:** Using a custom-modified HumanCoreExome array (Illumina), 678 patients of European descent recruited in five different clinics were genotyped. Response was defined as the change in best corrected visual acuity (VA) after the loading dose of three monthly ranibizumab or bevacizumab injections. An inverse normalization per sub-cohort was applied to the distribution of the phenotype to control for inter-clinic differences. After imputation and quality control steps, 6,464,434 variants with a minor allele frequency >5% were assessed for association with treatment response. Linear regression analysis was conducted adjusting for the first two principal components using EPACTS software.

**Results:** Suggestive peaks on chromosomes 2 and 18 were identified. The top associated variants had a  $p=2.27 \times 10^{-6}$  (chromosome 2) and  $p=3.83 \times 10^{-7}$

(chromosome 18), respectively. For the 34 known AMD lead variants, no variant showed significance to the 0.05/34 level.

**Conclusions:** We report the largest GWAS so far on response to anti-VEGF therapy for nvAMD. Replication of top variants may reveal novel genes influencing AMD treatment response.

### P15.04

#### Paradigm of arsenic exposure in normal and triple negative breast cancer cells

O. L. Braicu, A. Jurj, L. Raduly, O. Zanoaga, L. Policiuc, R. Cojocneanu-Petric, C. Braicu, I. Berindan-Neagoe;

Research Center for Functional Genomics, Biomedicine and Translational Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

**Introduction:** Arsenic was widely used in traditional breast cancer treatment and recently the interest for therapeutic implication has increased, particularly for triple negative breast cancer (TNBC), subtype of breast cancer with the lowest survival rate. In this study were evaluated cellular and molecular effects for arsenic using *in vitro* models.

**Material and methods:** We evaluated the effect of 50 nM arsenic in normal (HMEC cells) and TNBC breast cancer cells (Hs578T cells) by multiple methods, such as MTT assay, invasion using a matrigel approach and apoptosis by fluorescence microscopy and transcriptomic profile using Agilent microarray technology.

**Results:** Our results showed a reduced cell proliferation and invasion, the activation of apoptosis in a dose-response and time-dependent manner, especially in Hs578T cells as response to arsenic exposure. For HMEC cells we had 1398 downregulated genes and 153 overexpressed genes, meanwhile for Hs578T cells were 1223 downregulated genes and 648 overexpressed genes based on a fold-change cut-off of  $\pm 2$  and  $p\text{-value} \leq 0.05$ . For Hs548T cells the main altered canonical pathways were related to kinases and cAMP-mediated pathways and for HMEC cells were related to immune response, particularly IL-17 pathway.

**Conclusion:** Our data reveals a complex role for arsenic, in normal cells was observed a proinflammatory effect, via IL-17 pathway, recognised as a prone factor for breast cancer-associated inflammation. Strong evidence of antitumoral activity for arsenic was observed in Hs578T, via p53 and MAPK pathways, with implication in TNBC management, as alternative or in tandem with classical chemotherapy.

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### P15.05

#### Translarna™ (ataluren): A Novel Readthrough Drug for Treating Nonsense Mutation Genetic Disease

P. Trifillis<sup>1</sup>, E. Welch<sup>1</sup>, M. Weetall<sup>1</sup>, D. Wang<sup>2</sup>, K. M. Keeling<sup>3</sup>, D. M. Bedwell<sup>3</sup>, J. Rodriguez<sup>4</sup>, X. Luo<sup>1</sup>, G. Elfring<sup>1</sup>, H. Kroger<sup>1</sup>, P. Riebling<sup>1</sup>, T. Ong<sup>1</sup>, R. Spiegel<sup>1</sup>, S. W. Peltz<sup>1</sup>;

<sup>1</sup>PTC Therapeutics, South Plainfield, NJ, United States, <sup>2</sup>Horae Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA, United States, <sup>3</sup>University of Alabama at Birmingham, Birmingham, AL, United States, <sup>4</sup>PTC Therapeutics, Madrid, Spain.

Translarna is a novel, orally administered small-molecule drug that enables the ribosome to read through a premature stop codon, allowing the formation of a full-length protein. It therefore has the potential to treat any nonsense mutation (nm) mediated orphan genetic disease. In nm Duchenne muscular dystrophy (DMD), Translarna increased dystrophin protein in muscle (Phase 2a trial) and slowed disease progression (Phase 2b and 3). Clinical benefit of Translarna, coupled with a strong safety profile, led to its European approval for nmDMD. In patients with nm cystic fibrosis (CF), Translarna induced functional CFTR protein (Phase 2a trials). In a Phase 3 trial, Translarna increased %predicted forced expiratory volume compared to placebo in patients not receiving tobramycin inhalation, resulting in a 40% reduction in pulmonary exacerbation frequency. A second Phase 3 trial in nmCF is underway. Since ataluren crosses the blood-brain barrier, it can potentially treat neurologic symptoms associated with nm Mucopolysaccharidosis type I (MPS I) which are not treatable by enzyme replacement therapy. Ataluren reduced levels of glycosaminoglycans in the brain and multiple other tissues in a MPS I mouse model. Aniridia, a panocular developmental disease caused by mutations in the PAX6 gene, has no available treatment. Ataluren increased Pax6 protein in the retina and cornea and restored the iris in mice. Phase 2 trials in nmMPS I and nm aniridia are being initiated. nmCDKL5/Dravet has recently been selected as the 5th indication and selection of additional indications based on unmet medical need and other considerations is ongoing.

## P15.06

**A efficient and accurate end-to-end next-generation sequencing solution for identifying and interpreting disease causing variants in rare diseases**

**A. Joecker<sup>1</sup>, S. Shah<sup>2</sup>, B. Solomon<sup>3</sup>, J. Vockley<sup>3</sup>, G. Eley<sup>3</sup>, S. Tang<sup>2</sup>, B. Oester<sup>1</sup>, M. Hadjisavas<sup>2</sup>,  
<sup>1</sup>QIAGEN Bioinformatics, Aarhus, Denmark, <sup>2</sup>QIAGEN Bioinformatics, Redwood City, CA, United States, <sup>3</sup>Inova Translational Medicine Institute, Falls Church, VA, United States.**

Identification of causal variants in rare and undiagnosed diseases can be both challenging and time consuming, with a lot of time invested in aligning variant calling, annotation, and interpretation workflows or investigating false positive and non-disease-associated variants. The QIAGEN Bioinformatics Hereditary Disease solution delivers increased sensitivity for identifying causal variants, while shortening the list of candidates to follow-up. This high performance is achieved with a novel, streamlined, one step, end-to-end workflow that includes Biomedical Genomics Workbench, Ingenuity Variant Analysis, and HGMD, that takes sequencing reads to biological insight. The workflow fully integrates variant calling with the QIAGEN Knowledge Base and HGMD to provide a scalable production grade environment to enable efficient and accurate causal variant discovery. To evaluate the performance of this approach we analyzed 15 TRIOs from the INOVA clinic and several exomes and whole genomes from different clinical labs for which the causal variants are already known. In all cases we are able to identify the disease-causing variant, while reducing the number of variants to investigate, by 94% to 100% using this easy one-step solution.

## P15.07

**Breast cancer and predictive protein biomarkers in different cell lines to personalized drug design**

**S. Jeibouei, H. Zali;**

*Shahid beheshti university of medical sciences and health services, Tehran, Iran, Islamic Republic of.*

**Introduction:** As breast cancer accounts for about 30% of all cancers and is by far the most common type of cancer that affects women worldwide, and has heterogeneous patterns of protein expression, the resistance of cancer cells to chemotherapeutic drugs represents a major problem in treatment thereof. Analysis of proteomics data could be a new approach to targeted therapy of breast cancer. **Materials and methods:** In the present study we used data set containing glycoprotein expression of HER2 positive cell lines and ER positive cell lines to determine new predictive protein biomarkers related to drug resistance. Dataset of 180 glycoproteins in three HER2 positive cell lines, BT474, HCC1954, and SKBR3 in five ER positive luminal cell lines, BT474, HCC1428, MCF7, T47D, and ZR751 were selected and statistically compared. Network analysis was used to determine the important proteins based on the highest degree and betweenness centrality. **Results:** We found P07339, P20645, P11279 and P07602 were overexpressed in all HER2 positive cell lines. Furthermore, P05067 and P02751 had highly significant connectivity in glycoprotein network and high betweenness centrality proteins compared with others. Statistical comparison and network analysis in ER positive luminal cell lines showed that P07339, P07602, P13473 and P15586 were overexpressed and P05067 and P02751 had highly significant connectivity in glycoprotein network and the highest betweenness centrality compared with other proteins. **Discussion:** In addition to classical molecular index for these two breast cancer categories, we introduce new candidate predictive biomarkers to drug design and personalized medicine.

## P15.08

**Robust, sensitive and reliable analysis of formalin-fixed paraffin-embedded material using a single molecule molecular inversion probes-based cancer hotspot gene panel**

**A. Eijkelenboom, E. Kamping, A. Kastner-van Raaij, S. Hendriks-Cornelissen, R. Kuiper, A. Hoischen, M. Nelen, M. Ligtenberg, B. Tops;**  
*Radboudumc, Nijmegen, Netherlands.*

**Introduction:** Mutation and CNV analysis of tumour DNA to predict the best treatment options calls for a sensitive technique analyzing multiple targets simultaneously with short turn-around time. To obtain sufficient coverage depth usually PCR-based enrichment strategies are used to analyze routinely obtained formalin-fixed, paraffin-embedded (FFPE) tissue, fine needle aspirates or smears. As in contrast to capture-based techniques, these strategies do not allow assessment of the amount of unique template molecules analyzed. We evaluated a single molecule Molecular Inversion Probe (smMIP)-based strategy which precludes false negative results due to unrecognized amplification of a too low amount of template molecules.

**Materials and Methods:** A smMIP-based panel with (regions of) 26 genes that serve as predictive or differential diagnostic marker was designed and

evaluated on routine diagnostic tumour samples.

**Results:** The smMIP-based approach was able to detect all SNVs, small deletions and insertions (1-63 nt) and amplifications. Strand-specific amplification of both the sense and antisense strand allowed recognition of FFPE induced C>T artefacts. The merging of PCR-duplicates with identical 'single-molecule-tags' into consensus sequence reads provided true sequence coverage and eliminated the problem of PCR- and sequencing-artefacts allowing reliable mutation calling at only 3% mutant reads. The technique was robust minimizing the amount of rework and guaranteeing turn-around times of maximally 7 days.

**Conclusions:** smMIP-based library preparation combines the advantages of PCR- and capture-based enrichment strategies and fulfills all criteria for use in routine testing of FFPE tumours and small biopsies. It has replaced the PCR-based NGS method in our routine diagnostic workflow.

## P15.09

**Phenotype of Fabry disease in patients with mutations amenable to migalastat**

**D. Hughes<sup>1</sup>, D. G. Bichet<sup>2</sup>, D. P. Germain<sup>3</sup>, R. Giugliani<sup>4</sup>, R. Schiffmann<sup>5</sup>, W. R. Wilcox<sup>6</sup>, J. P. Castelli<sup>7</sup>, E. R. Benjamin<sup>7</sup>, N. Skuban<sup>7</sup>, J. A. Barth<sup>7</sup>, C. Fernandez Almendros<sup>8</sup>;**

<sup>1</sup>University College London, London, United Kingdom, <sup>2</sup>Hôpital du Sacré Coeur,

<sup>3</sup>University of Montreal, Montreal, QC, Canada, <sup>4</sup>Hôpital Raymond Poincaré (APHP),

<sup>5</sup>University of Versailles-St. Quentin en Yvelines (UVSQ), Garches, France, <sup>6</sup>Medical

<sup>7</sup>Baylor Research Institute, Dallas, TX, United States, <sup>8</sup>Emory University, Atlanta, GA, United States, <sup>7</sup>Amicus Therapeutics, Cranbury, NJ, United States, <sup>8</sup>Amicus Therapeutics, Madrid, Spain.

**Introduction:** Fabry disease (FD) is a lysosomal storage disorder caused by mutations in the  $\alpha$ Gal A gene. Migalastat, a pharmacological chaperone, binds to  $\alpha$ Gal A, increasing physical stability, lysosomal trafficking, and cellular activity. Study 011 (FACETS) enrolled 67 ERT-naïve FD patients; Study 012 (ATTRACT) enrolled 60 ERT-experienced FD patients. Based on Migalastat-Amenability assay, 50 patients from FACETS and 56 patients from ATTRACT studies had mutant forms of  $\alpha$ Gal A amenable to migalastat. Baseline disease severity and literature-defined phenotype were assessed.

**Materials and Methods:** 600 FD-causing mutations were expressed in HEK 293 cells and  $\alpha$ Gal A activity was measured in the presence/absence of 10  $\mu$ M migalastat. 268 amenable mutant forms were identified. Proportions of patients with FD-related involvement in  $\geq 2$  organ systems were determined and the phenotype (classic/nonclassic) of mutations assessed.

**Results:** In FACETS, all male patients and 88% of female patients with amenable mutations had FD manifestations in  $\geq 2$  organ systems, >90% of patients had elevated plasma lyso-Gb3, and, among mutations characterized in the literature, 60% of all patients had mutations associated with a classic phenotype; 87% of male patients had baseline  $\alpha$ Gal A <3%. In ATTRACT, 81% of patients had multiorgan system disease, and 36% had mutations associated with a classic phenotype. Overall, 68% of mutations amenable to migalastat identified to date in medical literature are associated with a classic phenotype.

**Conclusion:** A majority of Phase 3 patients with amenable mutations had multiorgan system disease and a genotype associated with a literature-defined classic phenotype.

## P15.10

**IFNL3 rs4803217 polymorphism and treatment outcome in patients with chronic hepatitis C**

**B. Świątek-Kościelna<sup>1</sup>, E. Kałużna<sup>1</sup>, D. Januszkiewicz-Lewandowska<sup>1,2,3</sup>, J. Rembowska<sup>1</sup>, I. Mozer-Lisewska<sup>4</sup>, I. Bereszyńska<sup>4</sup>, J. Wysocka-Leszczynska<sup>1</sup>, J. Wysocki<sup>5</sup>, J. S. Nowak<sup>6</sup>;**

<sup>1</sup>Department of Molecular Pathology, Institute of Human Genetics of the Polish Academy of Sciences, Poznań, Poland, <sup>2</sup>Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation, University of Medical Sciences, Poznań, Poland, <sup>3</sup>Department of Medical Diagnostics, Poznań, Poland, <sup>4</sup>Department of Infectious Diseases, University of Medical Sciences, Poznań, Poland, <sup>5</sup>Department of Preventive Medicine, University of Medical Sciences, Poznań, Poland.

**Introduction:** Polymorphisms near the interferon lambda 3 (IFNL3) / interleukin 28B (IL28B) gene are strongly associated with hepatitis C virus (HCV) elimination. Recently, the single nucleotide polymorphism rs4803217 within the 3' untranslated region of the IFNL3 gene has been proposed as a causal variant that affects HCV clearance by altering stability of the transcript. The aim of our prospective study was to examine the association between IFNL3 rs4803217 polymorphism and treatment outcome in chronic hepatitis C (CHC) patients.

**Materials and Methods:** The study included 196 CHC patients (HCV genotype 1a/1b) treated with pegylated interferon (IFN)  $\alpha$  and ribavirin. rs4803217 genotyping was performed using high resolution melting analysis. Results

were confirmed by DNA sequencing.

Results: The rs4803217 GG, GT and TT genotypes were found in 54 (27.55%), 107 (54.59%) and 35 (17.86%) patients, respectively. One hundred thirty-seven patients completed 48-week therapy. 39.4% of them achieved sustained virological response (SVR). Relapse (end of treatment response without SVR) was observed in 39 patients (50.6%). The GG genotype was significantly associated with an increased SVR rate (GG vs GT+TT; OR=11.13, 95% CI: 4.80-25.81, p=1.41x10-9) and decreased risk of relapse (GG vs GT+TT; OR=0.18, 95% CI: 0.06-0.50, p=0.0007). No significant associations were found between treatment outcome (SVR, relapse) and age, gender, HCV genotype, baseline viral load as well as biochemical and histopathological parameters.

Conclusions: Based on the results obtained, it can be concluded that rs4803217 polymorphism is a useful marker for predicting SVR and virologic relapse in IFN-based therapy of chronic hepatitis C.

### P15.11

#### Possible role of 82G/A polymorphism of RAGE gene in accelerated aging

Z. Verde<sup>1</sup>, C. Santiago<sup>1</sup>, P. Resano<sup>2</sup>, I. Sánchez-Hernández<sup>3</sup>, J. M. Rodríguez González-Moro<sup>4</sup>, N. Garatachea<sup>5</sup>, F. Bandrés<sup>6</sup>, A. Lucía<sup>1</sup>, F. Gómez-Gallego<sup>1</sup>,

<sup>1</sup>Universidad Europea de Madrid, Villaviciosa de Odón, Spain, <sup>2</sup>Hospital de Guadalajara, Guadalajara, Spain, <sup>3</sup>Hospital Carlos III, Madrid, Spain, <sup>4</sup>Hospital Gregorio Marañón, Madrid, Spain, <sup>5</sup>Universidad Zaragoza, Huesca, Spain, <sup>6</sup>Universidad Complutense, Madrid, Spain.

Introduction: Aging is a dynamic process in which its rate and subsequent longevity of an organism are dependent upon the balance between the reactive intermediates of normal cellular metabolism and the ability of the body to reduce these by-products. Environmental, genetic, and accidental factors have influenced the human life span. Advanced glycation end products (AGEs) may play an important role in the processes of physiological aging. Ageing and smoking may also contribute to the acceleration of the formation of AGEs. The aim of the present study was to investigate any differences in the frequencies of receptor for the advanced glycation end products (RAGE) 82G/A polymorphism in subjects aged >100 years and individuals suffer from respiratory illness (accelerated aging).

Material and Methods: RAGE 82G/A (rs2070600) was analyzed in a cohort of 126 subjects aged >100 (107 women and 19 men) and 158 individuals with pulmonary disease (29 women and 107 men). Genetic marker was determined by RT-PCR. A logistic regression analysis adjusted for gender was performed.

Results: Regression analysis showed differences between (RAGE) 82G/A polymorphism frequency in both groups (OR=23.8, 95% CI=12.5-45.4). The number of women was larger among long-living subjects than in the unhealthy group.

Conclusions: Our data provide evidence that 82G/A polymorphism appears to confer sensitivity to respiratory illness and are in accordance with the fact that the SNP 82G/A is associated with lower levels of circulating RAGE than 82 G/G genotype. The differences in the genetic regulation of inflammatory processes may influence the presence of aging disorders.

### P15.12

#### An ITPA variant as a pharmacogenetic marker of thiopurines toxicity in Crohn's disease patients

J. Salazar<sup>1,2</sup>, A. Altes<sup>3</sup>, M. Martínez<sup>1</sup>, E. del Río<sup>1</sup>, J. Gordillo<sup>1</sup>, J. Llaó<sup>1</sup>, M. Serés<sup>1</sup>, M. Baiget<sup>1</sup>, E. García-Planella<sup>1</sup>;

<sup>1</sup>Sant Pau Hospital, Barcelona, Spain, <sup>2</sup>CIBERER U705, Barcelona, Spain, <sup>3</sup>Fundació Althaia, Manresa, Barcelona, Spain.

Background: Thiopurine therapy is effective in Crohn's disease (CD) treatment, although 25% of patients will suffer toxicity. Polymorphisms in genes involved in thiopurines metabolism may affect toxicity. We aim to analyse the role of TMPT and ITPA functional variants as pharmacogenetic markers of thiopurines toxicity in CD.

Methods: Patients with CD treated with thiopurines and with TPMT enzyme activity levels >5 U/ml RBCs were included. We analysed 3 variants in the TPMT gene (c.238G>C, c.460G>A and c.719A>G) and 2 in the ITPA gene (c.94C>A and IVS2+21A>C). Genotyping was performed by real-time PCR.

Results: We included 109 CD patients. One hundred and four patients (95%) were treated with thiopurines in monotherapy. The mean follow-up period was 87.4 months (range 4-264). Thirty-three patients (30.3%) developed one adverse drug effect and 11 patients (10.1%) had more than one. The most frequent adverse events were lymphopenia (n=23, 21%), hepatotoxicity (n=15, 13.7%), nausea (n=9, 8.2%) and leucopenia (n=6, 5.5%). Forty patients (36.7%) discontinued thiopurine treatment because of toxicity. We found a statistical significant association between the IVS2+21A>C ITPA

variant and toxicity (OR=3.1 [95% CI, 1.18-8.24], p=0.022, in a dominant model). Toxicity was more frequent in patients carrying the C allele than in patients with an A/A genotype (55.6% vs. 35.4%). In addition, patients carrying the C allele had a higher risk of treatment discontinuation than homozygous A/A patients.

Conclusions: The IVS2+21A>C ITPA variant might be a predictor marker of thiopurines toxicity in CD patients with an intermediate or high TPMT activity.

### P15.13

#### Influence of c.-889T>G variant on promoter activity of CYP2C19 gene

M. M. Novkovic, L. B. Rakicevic, J. S. Kusic-Tisma, D. P. Radojkovic;

Institute of molecular genetics and genetic engineering, Belgrade, Serbia.

Introduction: CYP2C19 is an enzyme responsible for metabolism of about 10% of all clinically used drugs. Different variants in CYP2C19 promoter region influence transcription activity of the gene. Interindividual variability in CYP2C19 activity is responsible for pharmacokinetics, drug efficiency and adverse drug effects. In this study we examine the influence of -889T>G variant on CYP2C19 promoter activity.

Materials and methods: The 1705 bp of the promoter region of the CYP2C19 gene was cloned into pGL4.1 luciferase reporter vector and transfected in HepG2 cell line. To evaluate the basal activity of wild type and -889T>G promoters, dual luciferase assays were performed. Since *in silico* analysis revealed presence of glucocorticoid response element in the target region, we analyzed influence of dexamethasone as potent glucocorticoid agonist on both promoters.

Results: The basal activity of promoter containing -889T>G variant was about 33% lower in comparison to wild type promoter (p<0.05). Further, we observed difference in activity between these two promoter variants in response to dexamethasone treatment. Dexamethasone increased the activity of both promoter variants, however effect on -889T>G variant promoter was less noticeable.

Conclusion: Our results indicate that -889T>G variant decreases promoter activity of CYP2C19 gene. Dexamethasone differently influence the activity of wt and -889T>G promoter variants. Since, CYP2C19 c.-889T>G variant can contribute for interindividual variability in drug response, further characterization of this variant and evidence of transcription binding sites in this region are needed.

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### P15.14

#### Full-length and phased CYP2D6 variant genotyping using the PacBio RSII

H. Buermans, R. Vossen, Y. Anvar, S. White, H. Guchelaar, J. den Dunnen, J. Swen, T. van der Straaten;

Leiden University Medical Center, Leiden, Netherlands.

The Cytochrome P450 2D6 enzyme, encoded by CYP2D6, is among the most important enzymes involved in drug metabolism. Specific variants in the gene are associated with changes in the enzyme's amount and enzymatic activity, which determines the rate at which drugs get metabolized. Different technologies exist to determine these sequence variants, such as the Roche AmpliChip CYP450 GeneChip, Taqman qPCR or Next Generation Sequencing. However, sequence homology between several cytochrome P450 genes and pseudogene CYP2D7 impairs reliable CYP2D6 genotyping and phasing (haplotyping). The PacBio RSII sequencing platform produces a combination of long reads and high-quality consensus sequences, enabling accurate variant calling and haplotyping.

We sequenced CYP2D6 in 24 samples with 12 different, clinically relevant, haplotypes using the PacBio sequencer and obtained full-length, phased CYP2D6 sequence reads, enabling accurate variant calling and haplotyping. Unphased diplotypes, previously determined with the Roche GeneChip, were confirmed for 21 samples, including a duplication of one of the haplogroup sequences for three samples. Two samples, originally showing a single haplogroup sequence, were subsequently demonstrated to contain either a deletion or tandem duplication. In total 62 unique variants were detected across the 24 samples, as well as variants not previously been associated with the described haplotypes. To aid analysis using standard reference sequences and HGVS nomenclature, we have established a LOVD-powered CYP2D6 variant database and added all reference haplotypes and data reported here.

In conclusion, our genotyping approach produces reliable CYP2D6 genotype calls, and reveals additional information about phasing and copy-number variation.

**P15.15****Pharmacogenomics of membrane transporters and metformin response in Type 2 diabetic patients***S. Semiz<sup>1,2</sup>, T. Dujic<sup>2</sup>, Z. Velija-Asim<sup>2</sup>, A. Causevic<sup>2</sup>;**<sup>1</sup>International University of Sarajevo, Sarajevo, Bosnia and Herzegovina, <sup>2</sup>University of Sarajevo, Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina, <sup>3</sup>University Clinical Center of Sarajevo, Clinic for Endocrinology, Sarajevo, Bosnia and Herzegovina.*

**Introduction:** Genetic variations of several membrane transporters, including organic cation transporters 1 and 2, encoded by *SLC22A1/2* genes, are implicated in the highly variable response to metformin, a first-line drug used to treat newly diagnosed Type 2 diabetes mellitus (T2D). Additional variants of *SLC47A1/2* genes, encoding multidrug and toxin extrusion protein 1 (MATE1) and MATE2-K, have been also recently related to treatment outcomes.

**Materials and Methods:** Since previous studies were mostly performed in healthy subjects, here we analyzed several common variants of *SLC22A1/2* and *SLC47A1/2* genes in 92 newly diagnosed T2D patients. We also collected phenotype data including, but not limited to, levels of fasting glucose (FG), insulin (FI), HbA<sub>1c</sub> and anthropometric measures, prior to and 6 and 12 months post-metformin treatment.

**Results:** Our data demonstrated significant association of *SLC47A1* rs2252281 SNP with higher decrease of FI levels ( $p<0.05$ ) and lower HOMA-IR ( $p<0.05$ ) upon 6-month treatment. Interestingly, we showed that *SLC47A2* rs12943590 was associated with lower decrease of FI levels ( $p<0.01$ ) and higher decrease of HOMA-IR ( $p<0.01$ ) upon 6-month treatment in T2D patients, which is in line with the *in vitro* data showing that this SNP increases transporter activity resulting in decreased drug effect.

**Conclusions:** Our results indicated that both MATE genotypes were associated with the markers of insulin resistance, with variant carriers of MATE1 and MATE2-K having an increased and reduced levels of these markers post-metformin treatment, respectively. Thus, these findings suggest that MATE1/2 variants could play the key role in mediating insulin resistance and optimal response to metformin.

**P15.16****Assessment of EGFR mutations by digital PCR in peripheral blood of non-small cell lung cancer patients***L. Camacho<sup>1</sup>, A. Taus<sup>2,3</sup>, G. Piquer<sup>4</sup>, A. Hernández<sup>2</sup>, R. Longarón<sup>2,4</sup>, G. Martínez<sup>2,4</sup>, R.**Correa<sup>2,4</sup>, M. Vela<sup>2</sup>, C. Montagut<sup>2,4</sup>, J. Albanell<sup>2,4</sup>, E. Arriola<sup>1,3</sup>, B. Bellosillo<sup>1,2</sup>,**<sup>1</sup>IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Barcelona, Spain, <sup>2</sup>Hospital del Mar, Barcelona, Spain, <sup>3</sup>University of Southampton, Southampton, United Kingdom.*

**Background:** Assessment of molecular markers in circulating-free DNA is a novel approach to improve patient selection for precision medicine. Digital PCR (dPCR) is a promising tool that may provide the required sensitivity to accurately perform EGFR mutation detection in non-small cell lung cancer (NSCLC) patients. The aim of this study was to assess the detection of EGFR mutations in plasma samples from NSCLC patients.

**Methods:** Fifty-eight plasma samples from 25 EGFR mutant NSCLC patients were assessed for EGFR mutations by dPCR Quantstudio 3D using validated Custom TaqMan SNP Genotyping Assays specific for each mutation and compared to standard-of-care (SOC) EGFR testing in plasma samples by BE-AMing. Paired tumour biopsies were available for 28 samples. In addition 10 negative plasma controls from breast cancer patients were also tested by dPCR.

**Results:** Analysis by dPCR detected EGFR mutations in 54/58 plasma samples tested. Interestingly, in 4 cases in which no mutation was detected by SOC testing, dPCR provided a positive result. A significant correlation was found between dPCR and SOC results quantitative values ( $R= 0.835$ ,  $p<0.001$ ), and this high correlation was observed among all EGFR mutations tested. No EGFR mutations were observed in any of the negative plasma samples tested. Paired plasma and tumoural samples were available in 28 samples. EGFR mutations were detected in plasma in 23/28 patients by dPCR showing a sensitivity of 82.1 %.

**Conclusion:** Digital PCR is a sensitive technique for detecting EGFR mutations in plasma samples.

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**P15.17****Translating genomic findings into clinical genetics applications: experiences from our first 6 years.***D. Bick<sup>1,2</sup>, S. Levy<sup>1,2</sup>, R. Myers<sup>1,2</sup>, G. Cooper<sup>1,2</sup>, K. Strong<sup>1</sup>, S. Newberry<sup>1</sup>, B. Wilk<sup>1</sup>, A. Weborg<sup>1</sup>, G. Beard<sup>1</sup>, J. Harris<sup>1</sup>, L. Handley<sup>1</sup>, C. White<sup>1</sup>, W. Jones<sup>1</sup>, J. Kelly<sup>1</sup>, J. Anderson<sup>1</sup>, F. ShaterFerdosian<sup>1</sup>, G. Scharer<sup>3</sup>, D. Dimmock<sup>4</sup>, J. Tapper<sup>5</sup>, H. Jacob<sup>1,2</sup>, E. A. Worthey<sup>6,2</sup>,**<sup>1</sup>HudsonAlpha, Huntsville, AL, United States, <sup>2</sup>University of Alabama, Birmingham, AL, United States, <sup>3</sup>Children's Minnesota, Minneapolis, MN, United States, <sup>4</sup>Medical College of Wisconsin, Milwaukee, WI, United States, <sup>5</sup>Envision Genomics, Huntsville, AL, United States, <sup>6</sup>HudsonAlpha Institute, Huntsville, AL, United States.*

Despite significant advances in our understanding of the basis of disease, the cause underpinning most human disorders remains fully or partially unknown. Identification of molecular changes provides an opportunity to understand their role in disease, and in a clinical setting to apply that understanding to prevention, diagnosis, and treatment. The advent of genome-wide sequencing has altered how molecular changes are identified and has transformed Medicine. We have 6 years of experience in the field of Clinical Genomics gathered as Molecular Diagnostic Labs, Freestanding and Children's Hospital Genomic Medicine Clinics, and through large NIH funded research programs. Currently, we achieve a ~38% diagnostic success rate via WGS application in a population that has in general undergone significant prior MDx. This shows that WGS is in many cases superior to conventional tests. Of note we are finding cases where patients have more than a single genetic diseases. In such cases an unbiased WGS approach significantly impacts care by finding all or most variants contributing to disease. Application of WGS has identified causal variants that would not have been uncovered through application of WES. In this talk we will discuss the technological, infrastructure, and process advances that have allowed us to perform lower cost Clinical WGS at HiSeqX scale. We will also present notable clinical findings that have helped us define the boundaries of the utility of this approach. Finally we will discuss how we have refined the methods used to apply WGS to the practice of medicine over the last 6 years.

**P15.18****A multiplexed NGS solution to evaluate potential tumor immune response and objectively stratify tumors by histopathology from FFPE samples***A. Mongan<sup>1</sup>, W. Tom<sup>1</sup>, J. Zheng<sup>1</sup>, Y. Sun<sup>1</sup>, S. Rozenzhak<sup>1</sup>, G. Bien<sup>1</sup>, T. Yoshino<sup>2</sup>, W. Okamoto<sup>1</sup>, H. Nishikawa<sup>3</sup>, F. Hyland<sup>1</sup>, J. Godsey<sup>1</sup>,**<sup>1</sup>Thermo Fisher Scientific, South San Francisco, CA, United States, <sup>2</sup>Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, Japan, <sup>3</sup>Division of Cancer Immunology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Tokyo, Japan.*

The emergent T cell checkpoint therapy has offered durable and potential curative results for many NSCLC and melanoma patients. Nevertheless, fewer than half of subjects in trials exhibited positive response (Sharma, 2015). These results greatly illustrate the necessity for a better understanding of tumor microenvironment, tumor-lymphocyte interactions, and biomarkers that could predict drug sensitivity. In particular, PD-L1 expression has been reported to be a promising marker of response for PD-L1 therapies (Meng, 2015). However, standard methods for assessing PD-L1 protein level via immunohistochemistry have been shown to be inefficient and highly variable (McLaughlin, 2015) depending on selected antibodies, staining techniques, and site of tumors (Meng, 2015). Furthermore, as investigators are often also interested in measurements of many immune-related markers, a gene panel approach offers a convenient solution to objectively quantify expression levels. Here we describe the identification and characterization of a research panel of approximately 300 genes designed to evaluate the expression of immune checkpoint pathway, level of tumor infiltrating lymphocytes, T cell regulation, interferon signaling, and presence of various lymphocyte subsets. Our preliminary data shows that expression of the selected genes can stratify non-small cell lung cancer FFPE samples by histopathology. Furthermore, quantitative read out of the IFNg is clear and unambiguous for IFNg positive and negative controls. Gene expression was measured by NGS using Ion AmpliSeqTM technology. Technical replicates were found to have  $>0.99$  correlation among each other. Assays on this research panel\* were also found to be robust with respect to low input amount (1-10 ng RNA).

## P15.19

### Exome sequencing of severe Incontinentia Pigmenti phenotypes identifies frequent mutations in the cobalamin pathway

F. Fusco<sup>1</sup>, M. I. Conte<sup>1</sup>, A. Diociauti<sup>2</sup>, M. F. Branda<sup>1</sup>, M. B. Lioi<sup>3</sup>, M. El Hachem<sup>2</sup>, M. V. Ursini<sup>1</sup>;

<sup>1</sup>Institute of Genetics and Biophysics, Naples, Italy, <sup>2</sup>Dermatology Unit, Bambino Gesù Children's Hospital-IRCCS, Rome, Italy, <sup>3</sup>University of Basilicata, Potenza, Italy.

Incontinentia pigmenti (IP, MIM308300, 0.7/100,000) is an X-linked dominant neuroectodermal disorder, caused by NF-kappaB-Essential-MODulator (NEMO) gene mutation. The IP is always associated with skin defects while the other neuroectodermal tissue can be variably affected. Data from BBRMI Incontinentia Pigmenti Genetic Biobank at IGB Institute (IPGB, <http://www.igb.cnr.it/ipgb>) failed to reveal genotype-phenotype correlation in our IP cohort because of a high variable phenotype: the neurological alterations (mental handicap, epilepsy etc.) in 34% of IP cases (IP-CNS), did not correlate to specific NEMO mutations. We performed exome sequencing of 3 unrelated severe IP-CNS cases carrying the same loss of function NEMOdel4-10 mutation, followed by targeted sequencing of 141 candidate genes clustered in cobalamin/folate and fatty acids oxidation pathways, in a cohort of 40 cases, 20 with severe IP-CNS (mental handicap, epilepsy etc.) and 20 mild-IP (only skin defect). We found a high frequency of genetic variations in cobalamin/folate metabolism among the IP severe cases (p-value<0.05). Common variants in MTHFR, MTRR, CUBN, ABCD4 genes known to affect homocysteine metabolism and in ACACB gene were present in different combinations in 16 IP severe (80%) and in singly in four control subjects (20%). Moreover, among the damaging variants we found a previously described mutation in CPT2 and a new MTRR mutation, each in a single IP-CNS case. This implicates cobalamin/folate gene variants as risk factor for severe IP-CNS in the presence of the common NEMO deletion. These genetic variants are relevant biomarkers for clinical validation that will lead to the development of new targeted therapies for IP.

## P15.20

### Analysis of common MDR1 (ABCB1) genes C1236T and C3435T polymorphisms in patients with chronic myeloid leukemia (CML) in treatment with imatinib mesylate

E. P. Silveira-Lacerda, L. C. G. Pereira, C. A. S. T. Vilanova-Costa, A. P. Barbosa, E. C. Castelli, L. P. Felicio, R. S. Tavares;  
Federal University of Goias, Goias, Brazil.

The multi-drug resistance 1 (MDR1) gene encodes for a P-glycoprotein (PGP), which acts against various kinds of xenobiotics. Several single nucleotide polymorphisms (SNPs) in this gene that may influence PGP level and function have been identified. The aim of this study was to analyze the frequency and prognosis of MDR1 SNPs, C3435T and C1236T, in the patients with Chronic Mieloid Leukemia (CML) in treatment with Glivec, to determine the significance of these SNPs with the clinical pharmacokinetics of oral Imatinib front of the treatment and to compare the results with other ethnic groups. We made genotype and haplotype analyses of the MDR1 gene in 96 CML patients. Genomic DNA was extracted and was analyzed by PCR-RFLP. Of the 96 CML samples, 31 were (CC), 13 (TT) and 52 (CT) for exon 12 (1236). For the exon 26 (3435), 35 were (CC), 12 (TT) and 49 (CT). All frequencies for both polymorphisms were in Hardy-Weinberg equilibrium (p=0.229 and q=0.414). We found percentage of association between polymorphisms and their distribution in different populations, and the response to treatment both cytogenetic and molecular difference was not statistically significant (p <0.05). We conclude that the observed allele frequency for exons 1236 were 59.4% for C and 40.6% for T and the frequencies for the exon 3435 were 62.0% for C and 38.0% for T. The relationship between the frequencies of polymorphisms of MDR1 in populations of different geographic locations, can provide tools that help in choosing a more appropriate and effective treatment of CML.

## P15.21

### Monitoring treatment response in EGFR mutant non-small cell lung cancer patients in peripheral blood

B. Bellosillo<sup>1,2</sup>, A. Taus<sup>1,2</sup>, L. Camacho<sup>2</sup>, A. Hernandez<sup>1</sup>, G. Piquer<sup>2</sup>, A. Dalmases<sup>1,2</sup>, G. Martinez<sup>1,2</sup>, R. Correa<sup>1,2</sup>, L. Pijuan<sup>1</sup>, D. Casadevall<sup>1</sup>, C. Montagut<sup>1,2</sup>, S. Serrano<sup>1</sup>, J. Albalanell<sup>1,2</sup>, E. Arriola<sup>2,3</sup>;

<sup>1</sup>Hospital del Mar, Barcelona, Spain, <sup>2</sup>IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Barcelona, Spain, <sup>3</sup>University of Southampton, Southampton, United Kingdom.

Background: EGFR mutation assessment is essential for deciding therapy of non-small cell lung cancer (NSCLC) patients. Mutation detection in circulating cell-free tumour DNA may be an alternative to tissue analysis at diagnosis and useful for tracking tumour clonal dynamics.

Methods: 112 plasma samples from 25 EGFR-mutant NSCLC patients treated with tyrosine kinase inhibitors (TKIs) were collected at baseline and along the course of the disease. EGFR mutations were quantified by BEAMing and/or digital PCR (dPCR).

Results: Comparison of 28 paired plasma and tumoural samples showed detection of EGFR mutations in plasma in 23/28 cases (8 cases M1a/M0; 15 cases M1b) showing a concordance of 82.1%. 4/5 plasma negative cases were M1a. 7 patients were analysed at radiological response to TKIs and showed undetectable EGFR mutation (n=5), decrease in mutant load (n=1) or increase (n=1). In one out of 2 responding patients with plasma samples previous to radiological evaluation, EGFR mutation was undetectable, being an early predictor of response.

Samples from 13 patients at radiological progression showed an increase in EGFR mutant load (n=5) or re-emergence of mutations (n=8). The p.T790M mutation of resistance was detected in 5/13 samples. In 4 cases, samples obtained prior to radiological progression, EGFR mutations reappeared (n=2) or increased (n=2).

Conclusion: A high association (82.1%) in the detection of EGFR mutations was found between plasma and tumour biopsies with BEAMing and/or dPCR. These technologies allow for early prediction of response and relapse and genetic characterisation of resistant disease in EGFR mutant NSCLC patients.

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## P15.22

### Clinical and molecular characterization of a novel INS mutation in patients with diagnosis of MODY

R. Artuso<sup>1</sup>, B. Piccin<sup>2</sup>, B. Mazzinghi<sup>3</sup>, A. Provenzano<sup>4</sup>, L. Giunti<sup>1</sup>, V. Palazzo<sup>4</sup>, S. Landini<sup>4</sup>, S. Ton<sup>2</sup>, S. Giglio<sup>1,4</sup>;

<sup>1</sup>Medical Genetics Unit, Meyer Children's University Hospital, Florence, Italy, <sup>2</sup>Tuscany Regional Centre of Pediatric Diabetes, Meyer Children's Hospital, Florence, Italy,

<sup>3</sup>Nephrology and Dialysis Unit, Meyer Children's University Hospital, Florence, Italy,

<sup>4</sup>Medical Genetics Unit, Department of Clinical and Experimental Biomedical Sciences, Mario Serio, University of Florence, Florence, Italy.

Maturity onset diabetes of the young (MODY) is a monogenic form of diabetes that accounts for 2-5% of all cases, frequently misdiagnosed as type 1 or 2 diabetes (T1D or T2D), whose symptoms are often overlapping. It is characterized by clinical and genetics heterogeneity with mutations in thirteen genes. In about 50% of MODY patients are not identified causative mutations.

We report a family with 4 members firstly diagnosed with T1D, in an age included between 5-10 years. A previous genetic test identified two heterozygous silent substitutions in HNF1α gene, according to which was made an ineffective attempt to stop insulin therapy, administering repaglinide and sulphonylureas. In order to clarify the genetic background and the reasons of treatment failure, we performed a target resequencing analysis investigating a set of 102 genes implicated in glucose metabolism. Interestingly, we identified in all affected members a novel INS gene mutation (c.125T>C). Until now 51 INS mutations have been identified, associated with a broad spectrum of clinical presentations including diabetes with severe neonatal onset and mild adult onset, suggesting allelic heterogeneity and distinct mechanisms underlying of the disease.

Our mutation is located in the insulin B-chain region recognized as important for insulin binding to its receptor, thus resulting in a significant impairment of insulin clearance. Our results broaden the spectrum of INS phenotypes and highlight the importance to study rare variants using NGS, with the aim to understand the molecular aetiology of diabetes and to provide a more personalised treatment for each genetic subtype.

## P15.23

### Prediction of methotrexate toxicity through the analysis of the single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene in children with acute lymphoblastic leukemia

E. M. Kataluña<sup>1</sup>, E. Strauss<sup>2,3</sup>, O. Zająć-Spsychata<sup>4</sup>, E. Gowin<sup>5</sup>, B. Świątek-Kościelna<sup>1</sup>, J. S. Nowak<sup>1</sup>, M. Fichna<sup>1</sup>, P. Mańkowski<sup>6</sup>, D. Januszkiewicz-Lewandowska<sup>1,4,7</sup>;

<sup>1</sup>Institute of Human Genetics of the Polish Academy of Sciences, Department of Molecular Pathology, Poznań, Poland, <sup>2</sup>Institute of Human Genetics Polish Academy of Sciences, Department of Functions of Nucleic Acids, Poznań, Poland, <sup>3</sup>Poznań University of Medical Sciences, Laboratory for Basic Research and Translational Medicine, Clinic of Internal and Vascular Surgery, Poznań, Poland, <sup>4</sup>Poznań University of Medical Sciences, Department of Oncology, Hematology and Bone Marrow Transplantation, Poznań, Poland, <sup>5</sup>Poznań University of Medical Sciences, Family Medicine Department, Poznań, Poland, <sup>6</sup>Poznań University of Medical Sciences, Department of Paediatric Surgery Traumatology and Urology, Poznań, Poland, <sup>7</sup>Department of Medical Diagnostics, Poznań, Poland.

Methotrexate (MTX) is a chemotherapy drug, which is commonly used to

treat acute lymphoblastic leukemia (ALL). Elimination and toxicity of MTX can be influenced by polymorphisms in genes encoding enzymes involved in its metabolism. The crucial role in MTX metabolism plays 5,10-methylenetetrahydrofolate reductase (MTHFR), encoded by the MTHFR gene. Therefore the aim of our study was to investigate the association between occurrence of C677T (rs1801133) and A1298C (rs1801131) polymorphisms in MTHFR gene and MTX-induced toxicity during treatment of children with ALL. We also examined the impact of simultaneous occurrence of analyzed polymorphisms in MTHFR gene on toxicity and elimination of MTX.

Genotyping of MTHFR polymorphisms was performed on DNA samples, isolated from PBMCs, of 47 children treated according to intensive chemotherapy for childhood ALL, ALL IC BFM 2009.

The occurrence of 677T-1298A haplotype was associated with prolonged MTX elimination and higher incidence of MTX-related toxicity. On the other hand, the occurrence of 677C-1298A haplotype had protective effect on MTX clearance and toxicity, that was not observed for 677C-1298C haplotype. In the case of coexistence of studied variants, for both 677CT/1298AC heterozygotes and 677TT/1298AA homozygotes toxicity incidents were more frequently observed.

The results obtained indicate the association between the MTHFR allele 677T and haplotype 677T-1298A and elevated risk of MTX-induced toxicity in pediatric ALL patients. Genotyping of C677T and A1298C polymorphisms of MTHFR gene may help to minimize the risk of MTX toxicities, hence it can be considered as a useful tool for individualization of the MTX-based chemotherapy.

#### P15.24

#### Association of pharmacogenetic variants with efficacy and toxicity in patients with osteosarcoma

H. I. Vos<sup>1</sup>, M. J. Coenen<sup>1</sup>, H. Gelderblom<sup>2</sup>, T. van der Straaten<sup>2</sup>, E. S. J. M. de Bont<sup>3</sup>, W. T. A. van der Graaf<sup>4</sup>, H. W. B. Schreuder<sup>4</sup>, L. C. M. Kremer<sup>4</sup>, F. N. van Leeuwen<sup>1</sup>, H. Guchelaar<sup>2</sup>, D. M. W. M. te Loo<sup>1</sup>;

<sup>1</sup>Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>4</sup>Emma Children's Hospital/Academic Medical Center, Amsterdam, Netherlands.

**Background:** Pharmacogenetics can be used to optimize treatment of patients with osteosarcoma. We previously identified genetic markers predictive of treatment outcome in genes of cisplatin and doxorubicin metabolic pathways. However, the complex metabolism of drugs used in osteosarcoma treatment involves a broader range of drug metabolic enzymes and transporters. Hence, we performed large scale screening of 1,936 genetic variants in 231 genes involved in drug metabolism and transport.

**Methods:** Germline DNA of two osteosarcoma patient cohorts (n=139 and n=177) treated with cisplatin and doxorubicin-based chemotherapy was genotyped using the DMET-Plus array and analyzed in a meta-analysis due to baseline differences for histological response and age. Associations between genetic variants and ototoxicity (SIOP grade 1-4) and 5-year Disease Free Survival (DFS) were assessed by logistic regression and Cox proportional hazards models respectively.

**Results:** 689 markers and 136 patients (cohort 1), and 669 markers and 174 patients (cohort 2) passed quality control. Upon meta-analysis, 16 markers in 14 genes were significantly associated ( $P<0.05$ ) with ototoxicity, including AOX1 encoding a protein involved in reactive oxygen species homeostasis. A total of 23 markers in 17 genes were associated with DFS. The genetic variant with the lowest p-value (0.007) was located in SLC22A14 and we confirmed the association between an ABCC5 variant and DFS. Validation of the findings is ongoing in 200 osteosarcoma patients.

**Conclusion:** We identified genes previously unknown to be related to cisplatin and doxorubicin metabolism and transport. Upon validation, these markers are of potential interest for optimizing therapy for osteosarcoma patients.

#### P15.25

#### Teaching Pharmacogenetics

C. B. García, M. Gómez, M. Uriel, M. P. Ribate;  
SAN JORGE UNIVERSITY, Zaragoza, Spain.

Pharmacogenetics is a recent science that studies how patients' genotype influences in the results of their drug treatments. It should be desirable that all kinds of health professionals involved in drug therapies of patients could know the importance and relevance of analyzing certain genes to optimize the effectiveness of the drugs or to avoid some adverse reactions.

The Vice-dean of Pharmacy Degree at San Jorge University recognizes a relevant paper of Pharmacogenetics in the academic training. Therefore, it has

been introduced as an optative subject in the healthcare orientation plan. Lecturers of this subject are not only geneticists but pharmacology and pharmacoeconomics lecturers to review important issues of these areas that complement the genetics point of view. Different professionals, mainly pharmacists, also collaborate with seminars for the students.

Besides, innovative teaching has been selected by the lecturers. It includes a review of one topic by each student (one gene or one drug therapy) that presents to the rest of the class. They also work in groups to solve a problem with real patients (with the same drug treatment but different results of effectiveness) by using a problem-based learning method.

Results of this educational project are that there are almost no failing grades among students and teacher's surveys are excellent.

If students have to develop part of Pharmacogenetics subject, analyze results shown by their colleagues and solve a problem with real patients, they are more involved with the subject and it is a more effective way to reach a significant learning.

#### P15.26

#### Pharmacogenetics in the clinics: Design and implementation of a customized pharmacogenetics genotyping array (PharmArray®)

P. Arias, I. Dapía, J. Tenorio, G. Gordo, M. Muñoz, A. Borobia, A. Carcas, P. Lapunzina;  
Medical and Molecular Genetics Institute (INGEMM), Madrid, Spain.

**Introduction:** The increasing evidence supporting the implementation of Pharmacogenetics (PhGx) information in the clinics as well as the development of pharmacoeconomic studies showing the cost-effectiveness of a preemptive approach should foster the transition to "personalized medicine" in the following years. In 2013 our group built a customized SNP-array (PharmArray®) which allowed genotyping of 192 SNPs previously related in the literature with drug-response. Basing in our experience these years and compiling recently described PhGx associations we have recently updated the original design to a second version of the platform.

**Materials and Methods:** For the SNPs selection process we decided to put together an in-house multidisciplinary team in order to achieve a good coverage of relevant PhGx associations in drug metabolism and transporter genes. After assessing many alternative genotyping platforms we finally chose the TaqMan OpenArray® technology as it resulted more suitable for the clinical approach in terms of simplicity of analysis, response times and economic expenses.

**Results:** The array includes 180 SNPs from 11 drug metabolism genes and 9 transporter genes with a relevant effect in the pharmacokinetic profile of some of the most frequently prescribed drugs these days. We also included some other genes with reported clinical relevance related to PhGx response.

**Conclusions:** To date the adoption of PhGx into the clinical practice has been challenging mainly due to economic reasons and certain prescribers' skepticism and discomfort. However in our experience, the implementation of this platform resulted cost-effective and provided useful information for prescribers and clinicians.

#### P15.27

#### Is pharmacogenetic-guided treatment cost-effective?

M. Verbelen<sup>1</sup>, M. E. Weale<sup>2,1</sup>, C. M. Lewis<sup>1</sup>;

<sup>1</sup>King's College London, London, United Kingdom, <sup>2</sup>Genomics plc, Oxford, United Kingdom.

**Introduction:** Pharmacogenetics has the potential to personalize pharmaceutical treatments and many pharmacogenetic associations have been discovered to date. However, for pharmacogenetic-guided treatment to be incorporated in standard health care it needs to be cost-effective as well as clinically beneficial.

**Methods:** We performed a review of economic evaluations for pharmacogenetic associations listed in the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>). We determined the proportion of publications that found pharmacogenetic-guided treatment to be cost-effective or dominant over the alternative strategies and we estimated the impact of free genetic testing on these conclusions.

**Results:** The literature search yielded 44 economic evaluations related to 10 drugs. Most of the 130 entries in the FDA table had no economic evaluation performed. Over half of the evaluations drew conclusions in favour of pharmacogenetic testing: the pharmacogenetic-guided strategy was cost-effective in 30% and even dominant (cost-saving) over the alternative option in a further 27% of analyses. In a setting where genetic testing is free, nearly three quarters of economic evaluations would support pharmacogenetic-guided treatment with 50% considering it dominant and 23% cost-effective. **Conclusions:** Pharmacogenetic-guided treatment can be a cost-effective and

even cost-saving strategy. Moreover, genetic tests are even more likely to be economically worthwhile if genetic testing was freely available on all individuals, which is a realistic future prospect. However, few drugs with pharmacogenetic associations have been studied and more economic evaluations are needed to support the uptake of genetic testing in clinical practice.

#### P15.28

#### Reporting of evidence for utility of pharmacogenomics: PGx for statins as an example

**T. Rigter**<sup>1,2</sup>, **M. E. Jansen**<sup>1,2</sup>, **W. Rodenburg**<sup>2</sup>, **S. W. J. Janssen**<sup>2</sup>, **M. C. Cornel**<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands,

<sup>2</sup>Centre for Health Protection, National Institute for Public Health and the Environment, Bilthoven, Netherlands.

**Introduction:** Advances from pharmacogenomics (PGx) have not been implemented into health care to the expected extent. Several barriers hamper this implementation, such as lack of information on test characteristics, financial hurdles and regulatory issues. One gap that will be addressed in this study is a lack of reporting on relevant outcome measures in literature to enable decision makers and clinicians to evaluate the clinical validity and clinical utility of PGx-tests.

**Methods:** A systematic review of current reporting in scientific literature was conducted on publications addressing PGx in the context of statins. 87 articles between 1950 and 2016 were included and information was selected on: study characteristics, reported outcome measures, and accompanying conclusions on potential clinical consequences.

**Results:** Most articles reported odds ratios as the preferred measure for the association between a genetic variant and drug response. Often conclusions on the implementation of a PGx-test were based on this odds ratio, without explicit mention of other measures or factors influencing the clinical validity and clinical utility, such as the number needed to test, the positive predictive value, the availability of alternative treatments, and the perceived impact of use of a test (on patient and/or public level).

**Conclusion:** Often authors report an odds ratio to illustrate the association between a genetic variation and outcomes, such as adverse drug responses or ineffective dosage. However, to be able to gain insight in the effect on a population and select effective tests, additional outcome measures are needed to estimate the clinical utility.

#### P15.29

#### Detection of relevant pharmacogenomic variants and CYP2D6 copy number using a highly multiplexed next generation sequencing assay

**F. Hyland, M. Manivannan, S. Chen, T. Chen, M. Anderson, D. Dewolf, T. Hartshorne, G.**

**Liu;**

**Thermo Fisher, South San Francisco, CA, United States.**

Cytochrome P450 enzymes metabolize about 75% of drugs, with UGT enzymes metabolizing another 15%. Variations in gene sequence or in copy number may result in an inactive, defective, unstable, low expressed, or absent enzyme, an increase in enzyme activity, or an altered affinity for substrates. Pharmacogenomic genes may predict whether an individual is a poor or rapid metabolizer, facilitating understanding of dose optimization and adverse drug reaction.

We designed a highly multiplexed pharmacogenomics (PGx) research panel to profile 137 variants and CYP2D6 copy number in a single amplification reaction using Ion Torrent sequencing. We include sample ID primers for sample discrimination and gender determination. High quality sequencing libraries were produced from as little as 10 ng of input DNA from archived buccal swab and cell lines.

We initially developed the Ion AmpliSeq pharmacogenomics research panel by sequencing 91 Coriell cell lines. Next, we sequenced hundreds of buccal swab samples from 5 different labs. We multiplexed 8 to 96 samples per chip and achieved high uniformity of sequencing depth. We compared the AmpliSeq panel genotypes to annotated genotypes of the samples, and to TaqMan OpenArray PGx assay. The study showed genotype concordance >99.8%; genotype reproducibility > 99.8%, and no-call genotype rate 98%, with a no-call rate below 2%.

This customizable research panel facilitates accurate genotyping and copy number determination of key pharmacogenomic variants.

#### P15.30

#### Correlation of PIK3CA mutations with clinicopathological features in breast cancer

**B. Kubová**<sup>1</sup>, **J. Šimová**<sup>1,2</sup>, **M. Uvírová**<sup>1,2</sup>, **J. Žmolíková**<sup>1,2</sup>, **S. Pitronová**<sup>1</sup>, **Z. Bravencová**<sup>3</sup>, **H. Tomášková**<sup>2</sup>, **I. Urbanovská**<sup>1,2</sup>, **L. Kalábová**<sup>1</sup>, **D. Konvalinka**<sup>1</sup>, **R. Měch**<sup>1</sup>, **R. Ondrušek**<sup>1</sup>, **I. Žebráková**<sup>1</sup>, **A. Hopenštová**<sup>1</sup>, **J. Dvořáčková**<sup>2,4</sup>;

<sup>1</sup>CGB laboratory Inc., Laboratory of molecular genetics and pathology, Ostrava, Czech Republic, <sup>2</sup>Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic, <sup>3</sup>Institute of Oncology, Faculty Hospital in Ostrava, Ostrava, Czech Republic, <sup>4</sup>Institute of Pathology, Faculty Hospital in Ostrava, Ostrava, Czech Republic.

**Introduction:** Breast cancer is the most frequently diagnosed cancer in female. Molecular aberrations in the phosphatidylinositol 3-kinase (PI3K) pathway have been documented across cancers. But their prognostic/predictive and therapeutic implications are still controversial. **Materials and Methods:** Molecular profiling was performed on 266 breast tumour DNA samples isolated from FFPE. PIK3CA and AKT1 mutations were determined by primer extension method. Correlations between PIK3CA mutations and clinicopathological features were estimated with the chi-squared test (95% CI). Relaps-free survival (RFS) rates, in the subgroup of HER2+ breast cancer patient treated with trastuzumab were calculated, based on the Kaplan-Meier method, and the curves were compared using the log-rank test. **Results:** Frequency of PIK3CA mutations were detected in 25,9 %. Mutation p.E17K in AKT1 gene was detected in 1,9 %. PIK3CA mutations were significantly associated with ER+ (p=0,0004), PR+ (p=0,004) and borderly significant with low histopathological grade (p=0,05). In the subgroup of 50 HER2+ patients treated with trastuzumab, better RFS was observed in PIK3CA wild-type patients compared with mutated tumours (p=0,0001). **Conclusions:** This study confirm high prevalence of PIK3CA mutations and their significant correlation with some clinicopathological characteristics. In the present study, patients with activating mutations in PIK3CA had a poorer outcome than PIK3CA wild-type cases. Currently there is no sufficient evidence to recommend routine genotyping of PIK3CA in clinical practice, however further data collection is required to draw a definitive answer. Supported by TE - The Technology Agency programme "Competence Centres", TE02000058, Center of competence for molecular diagnostics and personalized medicine.

#### P15.31

#### An open source web application for polygenic trait and disease risk prediction

**B. J. Vilhjalmsson**<sup>1,2</sup>, **U. Seren**<sup>3</sup>, **G. Athanasiadis**<sup>4</sup>, **G. Bhatia**<sup>2</sup>, **J. Cheng**<sup>1</sup>, **T. Mailund**<sup>1</sup>, **A. Borglum**<sup>4</sup>, **M. Nordborg**<sup>3</sup>, **M. Schierup**<sup>1</sup>;

<sup>1</sup>Bioinformatics Research Centre, Aarhus University, Aarhus, Denmark, <sup>2</sup>TH Chan Harvard School of Public Health, Boston, MA, United States, <sup>3</sup>Gregor Mendel Institute, Vienna, Austria, <sup>4</sup>Department of Biomedicine, Aarhus University, Aarhus, Denmark.

In recent years, predicting polygenic traits and disease risk from the genotype has become common practice in both clinical and non-clinical settings. Interpreting such predictions for individual genomes is nontrivial and results are typically obtained with proprietary software that is not publicly available. In non-clinical settings, 23andme and other private companies have led the charge in providing direct-to-consumer genetic testing, resulting in more than one million individuals being genotyped to date. This explosion of genetic data motivated us to develop an easy-to-install open source platform for polygenic trait and disease risk prediction. The platform is developed with both clinical and non-clinical settings in mind. It implements three main computational steps: 1) imputation, 2) ancestry inference, and 3) genetic prediction. Through careful choice of algorithms and efficient implementation, the whole pipeline can be applied to a 23andme genotype in about one minute, ensuring an interactive user experience for all of the three steps. To demonstrate how the application can be used in practice, we provide an online implementation (<http://thehonestgene.github.io>), where users can upload their 23andme genotypes and obtain polygenic predictions for height and BMI. The polygenic risk prediction is carried out using LDpred weights, which are trained on publicly available GWAS summary statistics. To estimate the accuracy of the predictions we used self-reported height and BMI from 609 Danish high school students as validation data. The prediction R2 in the sample after adjusting for age, sex, and the first ten PCs was 25% for height and 11% for BMI.

#### P15.32

#### Pharmacogenetic nexus between limb development and metabolic syndrome

**M. Krupkova, M. Janká, B. Chylikova, F. Liška, L. Šedova, D. Křenova, V. Křen, O. Šeda;**  
**Institute of Biology and Department of Medical Genetics 1st Faculty of Medicine, Charles University, Prague, Czech Republic.**

The polydactylous rat PD/Cub is an established rat model of metabolic syndrome and aberrant limb development manifesting as preaxial polydactyly.

We have previously identified a limited, 7-gene region on PD/Cub chromosome 8 affecting both conditions and isolated it on the background of spontaneously hypertensive rat in SHR.PD-(D8Rat42-D8Arb23)/Cub (SHR-Lx) congenic strain. The aim of our study was to assess the potential pharmacogenetic interaction between this PD-derived genomic region and all-trans retinoic acid (ATRA), a potent morphogen with evidence of metabolic effects.

The adult SHR and SHR-Lx male rats were administered ATRA (15 mg/kg/day) or vehicle for 16 days. The same protocol was repeated with animals undergoing administration of 1mg/kg ATRA on 13th day of embryonic development (13ED). All rats were subjected to comprehensive metabolic and morphometric profiling. In a separate experiment, effect of prenatal (13ED) ATRA administration on manifestation of polydactyly in SHR (n=118) and SHR-Lx (n=178) offspring was assessed.

We identified significant interactions of both prenatal and postnatal administration of ATRA with the differential segment in SHR-Lx congenic strain. The sensitising effect of the Lx was apparent for prenatal ATRA, affecting adiposity, HDL and small LDL cholesterol fractions, while postnatal ATRA affected significantly VLDL cholesterol and LDL triglycerides. SHR-Lx offspring developed front-feet preaxial polydactyly in 13.5 % cases; no such effect was observed in SHR.

The interaction of ATRA with chromosome 8 differential segment in SHR-Lx strain affects both the morphogenesis of the limb and the metabolic syndrome aspects, mimicking the effects observed in human conditions.

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#### P15.34

#### Accurate and cost-effective analysis of bi- and tri-allelic DME SNPs using target-activated rhPCR genotyping assays

D. Tsang<sup>1</sup>, S. Hansen<sup>1</sup>, K. Beltz<sup>2</sup>, Y. Wang<sup>1</sup>, Y. Bao<sup>1</sup>;

<sup>1</sup>Integrated DNA Technologies, Redwood City, CA, United States, <sup>2</sup>Integrated DNA Technologies, Coralville, IA, United States.

**Introduction:** Pharmacogenetic studies require a reliable method to test samples for inherited variations in drug metabolizing enzymes (DME) and associated transporter genes. Here we report a novel, target-activated, universal probe-based rhPCR genotyping assay for amplifying and detecting specific single nucleotide polymorphisms (SNPs), multi-nucleotide polymorphisms (MNPs), and insertion/deletions (InDels). This technology utilizes a Type II RNase H (RNase H2) in conjunction with a novel mutant Taq DNA polymerase to achieve highly specific allele discrimination and eliminate primer dimers. A universal reporter system was used to achieve a cost-effective genotyping solution, and enable multiple fluorophore detection of multi-allelic variants in a single reaction. This approach is particularly useful in pharmacogenetics where increasing numbers of tri-allelic SNPs are found to have pharmaceutical and clinical value.

**Materials and Methods:** In this study, we designed a collection of >150 assays for detecting potentially causative SNPs, MNPs, or InDels in DME genes, including tri-allelic targets. The assays were designed using an optimized assay design algorithm, ensuring high target specificity. All assays were tested with 137 Coriell DNA samples from three populations and synthetic templates representing each genotype.

**Result:** We successfully demonstrated >95% coverage for the desired targets (including tri-allelic SNPs), and >99% call rate and 100% accuracy were achieved for the tested samples.

**Conclusion:** Our target-activated rhPCR genotyping technology provides a cost-effective and highly specific genotyping solution. Multiplexing with multiple universal probes allows us to perform multi-allelic genotyping in a single reaction, thereby reducing the cost per genotype.

#### P15.35

#### e:Med - systems medicine network of Germany

S. Argo<sup>1</sup>, J. Erdmann<sup>2</sup>, K. Rippe<sup>3</sup>;

<sup>1</sup>e:Med Management Office, c/o German Cancer Research Center, Heidelberg, Germany,

<sup>2</sup>Institute for Integrative und Experimental Genomics, Universität zu Lübeck, Lübeck, Germany, <sup>3</sup>German Cancer Research Center (DKFZ) & BioQuant Center, Research Group Genome Organization & Function, Heidelberg, Germany.

**Introduction:** The e:Med ([www.sys-med.de](http://www.sys-med.de)) program aims at establishing systems medicine in Germany. e:Med promotes system-oriented research on diseases in order to develop improved prevention, more comprehensive diagnostics and individually adjusted therapy schemes in personalized medicine. The program brings together scientists with molecular-genetic, clinical, mathematical and information technology expertise, with the objective to advance research results into clinical applications. **Methods:** e:Med scientists systematically examine the complex interplay of pathophysiological

processes in the human body and with external factors by high-throughput 'omics' methods. This work is supported by the development of state of the art information technologies and data management tools. By recording and analyzing systems-wide data sets quantitative models on the disease state are developed. The resulting experimental and theoretical methods are translated into clinical applications. Results from the interdisciplinary e:Med research teams will be shown that work on cancer, neural and cardiovascular diseases and infection at 34 clinics and universities, 14 large research institutions and 6 industrial companies in 33 German cities, as well as at 3 universities outside Germany. The findings should contribute to an improvement in the design of clinical studies in the medium term. **Conclusion:** The nationwide e:Med research and funding concept advances systems medicine in Germany. e:Med is funded by the Federal Ministry of Education and Research (BMBF) since 2013.

#### P15.36

#### Maintenance therapy of childhood ALL patients induces TPMT gene expression in VNTR dependent manner

N. Kotur<sup>1</sup>, B. Zukic<sup>1</sup>, B. Stankovic<sup>1</sup>, K. Klaassen<sup>1</sup>, T. Karan-Djurasevic<sup>1</sup>, M. Ugrin<sup>1</sup>, M. Stojiljkovic<sup>1</sup>, N. Totic<sup>1</sup>, G. Nikcevic<sup>1</sup>, G. Milosevic<sup>2</sup>, L. Lazic<sup>2</sup>, D. Dokmanovic<sup>2</sup>, D. Janic<sup>2</sup>, S. Pavlovic<sup>1</sup>;

<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>2</sup>University Children's Hospital, Medical Faculty, University of Belgrade, Belgrade, Serbia.

Thiopurine S-methyltransferase (TPMT) is the most important enzyme involved in metabolism of thiopurine drugs, such as 6-mercaptopurine (6-MP). Great variability of TPMT activity cannot be explained considering just coding region variations. Variation of regulatory elements, such as VNTR architecture (i.e. number and types of repeats) in promoter, as well as chemotherapy drugs are shown to influence TPMT enzyme activity. However, it is not known whether these factors influence TPMT gene expression in ALL patients.

TPMT gene expression was measured in hematopoietic tissue of 57 childhood ALL patients, both before chemotherapy and during the maintenance therapy when 6-MP and methotrexate are administrate.

Our results show that maintenance therapy strongly induces TPMT expression, more than 3 times, on average. For each ALL patient, TPMT expression was higher during maintenance therapy, than before chemotherapy ( $p<10-10$ ). An interaction of maintenance therapy with VNTR region in TPMT promoter modified TPMT gene expression. Specifically, VNTR<sup>5a</sup>/\*<sup>5a</sup> carriers were found to be the highest expressers during the therapy ( $p=0.045$ ), even though they were low expressers before chemotherapy. Our results confirm negative correlation between "A" repeats number of VNTR and TPMT gene expression ( $rs=-0.35$ ).

Maintenance therapy strongly induces TPMT expression. This effect is modified by architecture of VNTR region of TPMT gene. It could be of great importance to consider TPMT genetic variations at the very beginning of the maintenance therapy for childhood ALL patients, especially for carriers of less expressed VNTR alleles.

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#### P15.37

#### A rapid one week exome sequencing analysis for neonatal intensive care

K. Neveling<sup>1</sup>, S. van den Heuvel<sup>1</sup>, D. Zafeiropoulou<sup>1</sup>, S. Castelein<sup>1</sup>, J. H. Schuurs-Hoeijmakers<sup>1</sup>, W. van Rens-Buijsman<sup>1</sup>, R. Pfundt<sup>1</sup>, E. J. Kamsteeg<sup>1</sup>, A. Simons<sup>1</sup>, A. R. Mensenkamp<sup>1</sup>, L. Snijders-Blok<sup>1</sup>, E. K. Vanhoutte<sup>2</sup>, T. J. van Essen<sup>3</sup>, K. Diderich<sup>4</sup>, C. Gilissen<sup>1</sup>, W. van Zelst-Stams<sup>1</sup>, R. Mathijssen<sup>1</sup>, M. R. Nelen<sup>1</sup>;

<sup>1</sup>Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Maastricht university medical center, Maastricht, Netherlands, <sup>3</sup>University of Groningen, Groningen, Netherlands, <sup>4</sup>Erasmus medical centrum Rotterdam, Rotterdam, Netherlands.

**Introduction:** Exome sequencing has been widely introduced in clinical laboratories. However, batching of samples to run high throughput sequencers efficient results in relatively long turnaround times (TATs). For specific patient groups such as new-borns in neonatal intensive care units (NICUs), these TATs need to be reduced dramatically in order to have impact on clinical decision making.

**Method:** Trio analysis is performed to assist interpretation and enables *de novo* mutation detection. The Sureselect QXT protocol (v5, Agilent) is used for library construction which is sequenced on a NextSeq500 (Illumina). Median coverage is 200-300x. Automated file handling allows rapid BWA mapping, GATK variant calling and annotation. The TATs are aimed at 5 to 7 days: 1½ days for DNA isolation and library construction, 29 hours of sequencing, and ±1 day each for data analysis and data interpretation.

**Results:** Five cases have been sequenced to date using this workflow: two having ID, two movement disorder cases, and one immune disorder case. In two causative mutations were identified. One ID case carried a disruptive frameshift mutation in the recently identified ID gene *TRIP12* whereas one movement disorder patient carried two mutations in the recessive gene *RARS2*, leading to pontocerebellar hypoplasia type 6. The residual samples remained negative. Time to report was 6 working days.

**Conclusion:** We demonstrate a rapid exome analysis workflow using Next-Seq500 sequencing. Turnaround times of 5-7 days are well within reach. Trio sequencing combined with high median coverage aids in rapid interpretation and successfully identified causative mutations in 2 of 5 patients.

## P16 Omics/Bioinformatics

### P16.01

#### European Genome-phenome Archive (EGA) - A secure archive for human genomic and phenotypic data

*J. P. Almeida-King<sup>1</sup>, D. Spalding<sup>1</sup>, J. Rambla<sup>2</sup>, M. Alberich<sup>2</sup>, A. Senf<sup>3</sup>, P. Arce Garcia<sup>1</sup>, A. Carreno-Torres<sup>2</sup>, J. Kandasamy<sup>1</sup>, G. Kerry<sup>1</sup>, O. M. Llobet<sup>2</sup>, A. Lloret i Villas<sup>2</sup>, M. M. Quaresma<sup>2</sup>, T. Smith<sup>4</sup>, S. de la Torre<sup>2</sup>, S. Ur-Rehman<sup>1</sup>, P. Flicek<sup>1</sup>, A. Navarro<sup>2</sup>, H. Parkinson<sup>1</sup>, <sup>1</sup>European Bioinformatics Institute, Cambridge, United Kingdom, <sup>2</sup>Centre for Genomic Regulation (CRG), Barcelona, Spain.*

The European Genome-phenome Archive (EGA) facilitates the secure storage and controlled distribution of genetic and phenotypic data, for the purpose of biomedical research. As of January 2016, the EGA securely stores over 3 petabytes of data derived from over 700K unique samples and spread across ~1600 distributable datasets.

Our collections include reference data for rare and common diseases, including data derived from the UK10K project, Human Induced Pluripotent Stem Cells Initiative (HipSci) and the International Cancer Genome Consortium (ICGC).

We work closely with the rare disease platform RD-Connect, facilitating the brokering of raw -omics data to RD-Connect partners, for the purpose of downstream variant processing and VCF file generation. The RD-Connect workflow takes advantage of our secure data streaming service, which has recently been updated to support on the fly file decryption-using FUSE layer technology, implemented to streamline processing pipelines.

Additionally, with over 1000 rare disease samples accessioned and stored at EGA, the new EGA REST API allows further integration of the sample metadata in EGA and the RD-Connect platform allowing full mapping and tracking of these data across the whole RD-Connect ecosystem. Continuing developments on submission processes, such as the REST API and submission portal will further enable RD-Connect and other consortia to integrate EGA into their pipelines without having to deal with security and privacy issues. The EGA is maintained by European Bioinformatics Institute (EMBL-EBI) and the Center for Genomic regulation (CRG) and is available at <http://www.ega-archive.org>.

### P16.02

#### Highly Contiguous de novo Human Genome Assembly and Long-Range Haplotype Phasing Using SMRT Sequencing

*S. Picton<sup>1</sup>, J. Ekhholm<sup>1</sup>, M. Ashby<sup>1</sup>, J. Chin<sup>1</sup>, L. Hon<sup>1</sup>, J. Harting<sup>1</sup>, P. Peluso<sup>1</sup>, D. Rank<sup>1</sup>, S. Kujawa<sup>1</sup>, J. Korlach<sup>1</sup>, Pacific Biosciences, Menlo Park, CA, United States.*

Outside of the simplest cases such as haploid bacteria, or inbreds, genomic information is not carried in a single reference per individual, but rather has higher ploidy for almost all organisms. The existence of two or more highly related sequences within an individual makes it extremely difficult to build high quality, highly contiguous genome assemblies from short DNA fragments.

We have here developed a new algorithm and software called FALCON that is a polyploid aware assembler for de novo haplotype reconstructions from SMRT® Sequencing data. We sequenced Craig Venter's well-studied genome at 85-fold coverage using long insert libraries (>20kb) as input for single-molecule sequencing. We then used the hierarchical genome assembly process (HGAP) to generate highly accurate pre-assembled consensus reads, which were fed into an overlap consensus assembler. FALCON was applied to enable a diploid representation of the genome while maintaining the relationship between the alternative alleles in the form of associate contigs. Altogether the genome was assembled into 3004 contigs where the longest contig was 34.6Mb in size.

In order to assess the quality of the assembly we looked at how efficiently

we captured the 4 Mb MHC region on chromosome 6. This region is known to be challenging since it is highly repetitive and polymorphic. Falcon successfully identified 20 significant structural variants between the homologous chromosomes proving that it is able to reconstruct highly heterozygous sequences yielding near-complete sequence of all homologous chromosome pairs.

### P16.03

#### An integrative analysis of genomic studies for the identification of the molecular network regions associated with autism spectrum disorders.

*E. Mosca<sup>1</sup>, M. Bersanelli<sup>2</sup>, R. Alfieri<sup>1,2</sup>, M. Landini<sup>1</sup>, N. Galluccio<sup>1</sup>, M. E. Raggi<sup>4</sup>, L. Villa<sup>4</sup>, M. Moleteni<sup>1</sup>, A. Bonfanti<sup>4</sup>, S. Camposeo<sup>5</sup>, A. Massaglia<sup>5</sup>, G. Castellani<sup>2</sup>, F. Ciceri<sup>4</sup>, A. Marabotti<sup>1,6</sup>, L. Milanesi<sup>1</sup>, A. Mezzelani<sup>1</sup>.*

<sup>1</sup>Institute of Biomedical Technologies, National Research Council, Segrate, Italy,

<sup>2</sup>Department of Physics and Astronomy, University of Bologna, Bologna, Italy, <sup>3</sup>INGM, National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Milan, Italy,

<sup>4</sup>Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini (LC), Italy, <sup>5</sup>Scientific Institute, IRCCS Eugenio Medea, Brindisi, Italy, <sup>6</sup>Department of Chemistry and Biology, University of Salerno, Fisciano (SA), Italy.

**Introduction:** Molecular interaction networks are crucial for the interpretation of genome-wide data of pathologies characterized by a marked genetic heterogeneity like Autism Spectrum Disorders (ASDs). Indeed biological functions involved in a specific pathology are likely to be more conserved than individual genetic variations. We define the network regions enriched in genetic variations associated with ASDs. Compared with previous studies, we consider several reported evidences and the whole network topology to predict relevant interacting partners of ASDs genes.

**Materials and Methods:** Lists of genes associated with ASDs were collected from a manually curated database and genomic studies. A diffusion-based method was used to identify genes in network proximity to those from the lists. Direct and indirect protein-protein interactions were collected from five different databases. The Italian cohort (195 cases and 87 controls) was genotyped with a high-density platform.

**Results:** We defined genome-wide scores summarizing the proximity of each gene to the gene lists associated to ASDs. We highlighted the network regions, with corresponding biological functions, differently represented in the considered genomic studies. We found significant number of genes in network proximity to genes associated with ASDs among the genes reported in the literature as having a minimal evidence of association with ASDs and the genes containing alleles associated with ASDs in our Italian cohort.

**Conclusions:** We summarise the network regions densely populated by ASDs associated genes providing a network-based tool to quantify the functional relation of novel genes with those already associated with ASDs.

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### P16.04

#### Enforcement of consistent implementation of standards and guidelines for reporting of CNV and SNV events through software automation

*A. Venier<sup>1</sup>, A. O'Hara<sup>2</sup>, V. Wasnikar<sup>2</sup>, S. Shams<sup>2</sup>,*

<sup>1</sup>BioDiscovery, Stevenage, United Kingdom, <sup>2</sup>BioDiscovery, Hawthorne, CA, United States.

Recommended standards and guidelines for clinical case review for copy number variations (CNVs) and single nucleotide variants (SNVs) have been published by several European, American, and individual national genetic and cytogenetic associations which include ESHG, ECA and ACMG. These guidelines provide means to classify variants into different well-defined classes (e.g. Pathogenic, Benign, etc.). Integrated into the NxClinical software system is the Variant Interpretation Assistant (VIA), which uses a decision tree machine learning approach to set up rules for automatic pre-classification of events to be reviewed and interpreted by laboratory personnel. The system allows users to embed guidelines for consistent reporting. Benign, likely benign, pathogenic and likely pathogenic regions are pre-classified based on public copy number polymorphism databases and publications, including ClinGen, OMIM, and DECIPHER, and on previous in-house case classification results. VIA tracks results in real time from previously annotated samples to allow for interpretation of newly uploaded samples. Additionally, the system allows for multiple review and edits, as needed, with extensive user-based audit trailing. A set of previously reviewed samples with a mix of benign and pathogenic classified calls were imported into the system. VIA then pre-classified the samples and the results were compared. The system was able to correctly identify pathogenic results in known affected samples while finding no pathogenic results in known normal samples. VIA is capable of quickly and reliably classifying and reporting CNV and SNV

events while adhering to the standards recommended by ESHG, ECA, ACMG and national guidelines.

#### P16.05

#### Bioinformatic analysis of mRNA expression on apoptotic and autophagy genes shows changes in biological pathways involved in lung diseases

R. Al-Ali<sup>1,2</sup>, J. de Las Rivas<sup>3,2</sup>, R. González Sarmiento<sup>1,3,2</sup>,

<sup>1</sup>University of Salamanca - Faculty of Medicine-Department of Medicine, Salamanca, Spain, <sup>2</sup>Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain,

<sup>3</sup>University of Salamanca-Cancer Research Center/ CSIC (CIC-IBMCC), Salamanca, Spain.

Several genes were reported to have splicing variants (isoforms) that have contradictory effects. The question that we tackle is the extent of these isoform-diseases, specifically in lung related illnesses. In order to be able to analyze the data from biological point of view, we focus on autophagy and apoptosis, two important pathways with known controversial changes in cancer and chronic obstructive disease (COPD).

We have chosen 87 RNA-Seq samples from ENA/EBI (European Nucleotide Archive), all were via Illumina technologies. The samples are from lung tumors (NSCLC), COPD lung tissue and normal lung tissue. We analyzed the samples using Tophat/Cufflinks on Galaxy platform, with quality trimmer when needed. We selected a panel of 60 genes relate to autophagy and apoptosis, and studied gene and isoform expression. Our study evaluated each gene and isoform in face of both software misinterpretation and biological significance.

We were able to track the sequence of mRNA expression and analyze the theoretical effect on the pathways they are involved in. Our results show that changes in the expression of caspases isoforms are able to turn the pathway from non-apoptotic to anti-apoptotic and vice-versa. While the main isoform change in autophagy pathway was present in mTOR. Those changes could be involved in development of cancer and COPD.

Our study shows that RNA-Seq data can be used to track a sudden change in biological pathways if treated with previous biological knowledge, and that further improvement on software analysis and data interpretation is due.

#### P16.06

#### GeVaCT - Genomic Variant Classifier Tool

D. Daneels<sup>1</sup>, I. Grau<sup>2,3</sup>, D. Sengupta<sup>2</sup>, M. Bonduelle<sup>1</sup>, D. M. Farid<sup>2</sup>, D. Croes<sup>1</sup>, A. Nowé<sup>2</sup>, S. Van Dooren<sup>1</sup>;

<sup>1</sup>Centre for Medical Genetics, Reproduction and Genetics, Reproduction Genetics and Regenerative Medicine, Vrije Universiteit Brussel (VUB), UZ Brussel, Brussels, Belgium,

<sup>2</sup>Como - Artificial Intelligence Lab, Vrije Universiteit Brussel (VUB), Brussels, Belgium,

<sup>3</sup>Department of Computer Sciences, Universidad Central de Las Villas, Santa Clara, Cuba.

High throughput screening (HTS) techniques, like mendeliome, whole exome and genome screening, are becoming a routine in a clinical diagnostic setting. However, classifying the identified genomic variants as benign or (likely) pathogenic, is still a tedious and time consuming process for the (clinical) geneticist. To facilitate this variant classification process, we have developed GeVaCT, a standalone Java based tool that implements and automatizes a published variant classification scheme for autosomal dominant disorders. GeVaCT currently supports annotated variant files from Alamut Batch (Interactive Biosoftware), with future plans to support input from other variant annotation tools.

The variant classification process currently implemented in GeVaCT is based on a published scheme in the context of cardiac arrhythmias (Hofman et al., 2013). The implemented scheme consists of two phases: pre-processing and variant classification. During pre-processing, the annotated variant file from Alamut Batch is imported and filtered based on the presence of the variant in databases with described variants or a local database, the variant location, the coding effect and the variant allele frequency in an ethnically matched population. The variant classification workflow depends on the type of variant: either missense or nonsense/frame-shift. Each attribute used gets a weighted score that is summed up with the others to come to a first variant classification. This first score is updated based on familial and functional information obtained for the variant-of-interest. The final result is a classification of the variant in one out of five classes ranging from non-pathogenic to pathogenic.

#### P16.07

#### Application Specific Barcoding Strategies for SMRT Sequencing

R. Vogelsang;

Pacific Biosciences, Menlo Park, CA, United States.

Over the last few years, several advances were implemented in the PacBio® RS II System to maximize throughput and efficiency while reducing the cost per sample. The number of useable bases per SMRT® Cell now exceeds 1

Gb with the latest P6-C4 chemistry and 6-hour movies. The new SMRT Cell yields could be in excess relative to project needs for applications such as microbial sequencing, targeted sequencing, Iso-Seq™ full-length isoform sequencing and Nimblegen's target enrichment. To this end, barcoding is a viable option for multiplexing samples.

Here, we present specific examples on strategies and best practices for multiplexing samples for five different applications using SMRT Sequencing. For microbial samples we studied the performance of 2- to 8-plex sequencing of *H. pylori* stains using modified SMRTbell™ adapters. For full-length amplicon sequencing we used barcoded adapters to multiplex five Class I and Class II HLA genes (3.3 - 5.8 kb) for 96 patients. For Iso-Seq full-length cDNA sequencing, RNA samples from 6 maize tissues were multiplexed to generate barcoded cDNA libraries. The NimbleGen SeqCap Target Enrichment method, combined with PacBio's long-read sequencing, provides comprehensive view of multi-kilobase contiguous regions, capturing both exonic and intronic regions. Here we successfully multiplexed 12 samples using the Nimblegen Neurology panel.

#### P16.08

#### The workflow system based on Hadoop for bio-big data analysis

P. Kim, G. Ko, J. Yoon, B. Lee, R. Kim;

Korean Bioinformation Center, KRIBB, Daejeon, Korea, Republic of.

Because of the exponential growth of biological data since the introduction of next generation sequencing technology, the analysis of massive bio data became a more complicated and difficult problem. To find out meaningful information from these massive data, researchers need IT skills to compose and run complicated bioinformatics analysis processes which are constituted many open-source programs. IT infra such as computing servers, network devices, and storages is also essential to run analysis pipelines for massive data. To address this problem, we developed BioExpress which provides cloud service for massive bio data. BioExpress consist of two systems: OpenBio and CLOSHA. OpenBio is hybrid cluster system which can run Hadoop and Linux programs. It is based on HDFS which can run not only Hadoop programs but also Linux programs applying the disk caching technique which transfers a file in HDFS into general Linux file system in each request. This system is economic than general cluster system with shared storage because it is based on HDFS which is cheaper than shared storage. BioExpress also supports parallel processing feature using MapReduce in Hadoop. CLOSHA is an automatic workflow-modeling system that enables researchers to represent the process of bio-data analysis as a workflow which is composed of a sequence of analysis tools by connecting the output of preceding tool and the input of following tool in sequence, with same formats. Users can easily analysis the complicated and massive bio-data through CLOSHA.

#### P16.09

#### Combining multiple strategies for prioritizing variants using exome sequencing data in familial Meniere's disease and autosomal diseases

T. Requena<sup>1</sup>, A. Gallego-Martinez<sup>1</sup>, L. Freijo<sup>1</sup>, C. Martin-Sierra<sup>1</sup>, J. A. Lopez-Escamez<sup>1,2</sup>,

<sup>1</sup>Otolaryngology & Neurotology Group CTS495, Department of Genomic Medicine, GENYO -

Centre for Genomics and Oncological Research - Pfizer/University of Granada/Junta de Andalucía, PTS, Granada, Spain, <sup>2</sup>Department of Otolaryngology, Complejo Hospitalario Universidad de Granada (CHUGRA), Granada, Spain.

**Introduction:** The identification of disease-causing variants in autosomal dominant (AD) diseases using exome-sequencing data remains a difficult task in small pedigrees. We describe a combined strategy using multiple variants prioritization tools, and phenotype ontology for identifying disease-causing variants in AD diseases tested in a) familial Meniere's disease (FMD), b) AD sensorineural hearing loss (AD-SNHL) and c) central nuclear myopathy (CNM).

**Materials and Methods:** Three methods (Pathogenic Variant -PAVAR score, Variant Annotation Analysis and Search Tool -VAAST + Phevor and Exomiser-v2) were compared in FMD exome-sequencing datasets. The effectiveness to filter and prioritize causal variants in small pedigrees was validated in silico using variants described in HGMD for AD-SNHL and CNM.

**Results:** The combination of prioritizing methods in exome datasets from small pedigrees in FMD can filter the variants to a reduced number of candidates ( $4.6 \pm 2.1$ , ranked in the top 20 by each method). Benchmarking analyses of the AD-SNHL and CNM show that Exomiser-v2 and VAAST+Phevor tools ranked variants of HGMD in the top 20 in more than 90% of the simulations, illustrating that only deleterious variants with a possible phenotype association remained. Selecting and combining the top 20 ranked variants by the three methods yielded a high predictive performance (AUC=0.823). **Conclusions:** Our results demonstrate that the combination of multiple vari-

ant-prioritization algorithms provides an effective method to filter and rank heterozygous variants in AD diseases despite the observed genetic heterogeneity in AD diseases.

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## P16.10 Raising awareness of clinical bioinformatics - can we MOOC a difference?

**A. C. Davies, F. Hooley, K. O'Malley, A. Brass;**  
The University of Manchester, Manchester, United Kingdom.

Within healthcare genomic sequencing approaches are generating huge quantities of data, requiring Clinical Bioinformaticians to design the IT infrastructure, data governance and pipelines to allow the analysis of these genomic data sets. They have an important responsibility for interrogating its quality to ensure the best possible outcomes for patients. A skill set currently desperately lacking in the UK.

Massive Online Open Courses (MOOC) are a popular and accessible way to educate large numbers of people, now expanding to include many more health focussed topics. In late 2015 we at The University of Manchester set out on a journey to write, produce and deliver the first MOOC aimed at raising the awareness of this new discipline to healthcare professionals: "Clinical Bioinformatics – Unlocking Genomics in Healthcare".

Over the past 3 years we have been delivering the Masters programme in Clinical Bioinformatics to educate and train trainee Clinical Scientist Bioinformaticians, the first cohort will graduate in summer 2016.

The MOOC is hosted on the FutureLearn platform, based on a social media approach, it allows learners to follow one another if an interesting discussion thread develops and also encourages peer review of co-learners work. We have worked with Clinical Geneticists and Clinical Bioinformaticians within the Manchester Centre for Genomic Medicine to include clinical case studies for learners to study. Here we will describe the platform, educational ethos and our learning journey to final production.

The MOOC can be found: <https://www.futurelearn.com/courses/bioinformatics/1>

## P16.11 Screening of thrombophilia-related phenotypes and pathways in a gene expression family study

**S. Picart-Armada<sup>1</sup>, A. Ziyatdinov<sup>2</sup>, A. Martínez-Perez<sup>2</sup>, A. Viñuela<sup>3</sup>, J. Souto<sup>2</sup>, E. Dermitzakis<sup>3</sup>, A. Perera<sup>4</sup>, J. Soria<sup>2</sup>, A. Buil<sup>4</sup>;**  
<sup>1</sup>Universitat Politècnica de Catalunya, Barcelona, Spain, <sup>2</sup>Institut d'Investigació Biomèdica, Sant Pau, Barcelona, Spain, <sup>3</sup>Department of Genetics Medicine and Development, University of Geneva, Geneva, Switzerland, <sup>4</sup>Mental Health Center St. Hans, Roskilde, Denmark.

Introduction: Family studies offer an opportunity to assess the inherited and acquired nature of the quantified traits. Here, we analyse phenotypes related to thrombophilia by partitioning the explained variance into a genetic component, a transcriptomic component and the residual effect.

Materials and methods: Our data corresponds to the GAIT2 project on idiopathic thrombophilia, whose cohort includes 935 individuals stemming from 35 pedigrees. 481 quantitative phenotypes were measured at time of recruitment, including anthropometric measurements, hemogram, hemostasis traits, as well as phenotypes related with platelet activity, homocysteine metabolism, inflammation and flow cytometry. We also sequenced the mRNA transcriptome from whole blood.

We perform variance component analysis on linear mixed effect models that explain the phenotypes in terms of the kinship structure and the gene expression. Additionally, we extend these models conceiving a pathway enrichment technique that tests whether including the gene expression similarity based on a particular pathway improves the explained variance in the phenotype.

Results and conclusions: On one hand, models involving kinship and expression recover previously reported heritability estimates for known phenotypes. Furthermore, their error term decreases by introducing expression in the models, therefore explaining former residual variance in terms of ambient factors. On the other hand, relevant pathways improve specific phenotype models, such as platelet activation explaining platelet abundance in blood.

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## P16.12

### Gibbon-human chromosomal breakpoints show a distinctive epigenetic signature

**N. Lazar, K. Nevonen, L. Carbone;**  
Oregon Health & Science University, Beaverton, OR, United States.

Chromosomal rearrangements are large-scale mutations that generate drastic structural changes in the genome. These events occur throughout evolution and are a hallmark of cancer. Despite their biological relevance, their mechanistic origins are poorly understood. While the study of chromosome fragility has been mainly focused on the sequence and architecture of chromosomal breakpoints, we hypothesize that other factors, above all epigenetic conditioning, should be considered.

In particular, we are using the gibbon genome as a model, as this species displays an unusually high occurrence of evolutionary chromosomal rearrangements and we have characterized the position of chromosomal breakpoints at very high resolution. We are employing a comprehensive strategy to explore the epigenetic landscape surrounding gibbon-human synteny breakpoints. This includes measuring DNA methylation, CTCF binding, histone marks and chromatin conformation (HiC-seq) in gibbon and five additional primate species and correlating these data through bioinformatics analysis.

Through this integrated analysis, we discovered that the epigenetic landscape of gibbon breakpoint regions is distinct from other genomic regions and remarkably conserved when compared to orthologous regions in other primates, indicating that the epigenetic state of breakpoints preceded gibbon speciation and was preserved after the genome reshuffling. Surprisingly, we find that the two sides of the breakpoints noticeably differ from each other in the distribution of DNA methylation rates and CpG density. This lack of chromatin homogenization could indicate a selection for chromosomal topological domains driven by functional effects. This is a remarkable finding given the recent and extensive reshuffling of the gibbon genome.

## P16.13

### TruePrime™ liquid biopsy: Tuning whole genome amplification towards improving the sensitivity of circulating tumor DNA analysis

**Á. J. Picher<sup>1</sup>, L. Blanco<sup>2</sup>, T. Caldés<sup>3</sup>, K. Pantel<sup>4</sup>, A. Schneider<sup>5</sup>;**  
<sup>1</sup>Sygnis Biotech SLU, Madrid, Spain, <sup>2</sup>Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain, <sup>3</sup>Hospital Clínico San Carlos, Laboratory of Molecular Oncology, Medical Oncology Department, Madrid, Spain, <sup>4</sup>University Medical Center Hamburg-Eppendorf, Department of Tumor Biology, Center of Experimental Medicine, Hamburg, Germany, <sup>5</sup>SYGNIS Bioscience GmbH & Co KG, Heidelberg, Germany.

TruePrime™ is the name of a novel technology dedicated to the amplification of genomic DNA. While the current gold standard MDA (multiple displacement amplification) relies on short oligonucleotides to start off the amplification, TruePrime™ is based on a combination of Phi29 DNA polymerase with the recently discovered primase/polymerase TthPrimPol. In this setup, TthPrimPol synthesizes the DNA primers needed for Phi29 DNA pol in the course of the reaction, which allows for the exponential amplification of genomic DNA. Key advantages of the TruePrime™ technology for amplification of single cell genomes include complete absence of primer artefacts, superior sensitivity down to the femtogram range, high reproducibility, little bias in genome coverage, and superior variant detection. Moreover, the TruePrime™ workflow is easy and reaction products work well with major NGS platforms. We are currently tuning the TruePrime™ technology towards the amplification of cell free DNA from plasma and serum with the intention to improve sensitivity and reliability of the method. Results on the feasibility of this approach will be presented.

## P16.14

### Low frequency variant detection from FFPE and cell-free DNA using target capture and molecular tagging

**M. Light<sup>1</sup>, S. Groenewold<sup>2</sup>, M. McNeill<sup>2</sup>, M. Jarosz<sup>1</sup>;**  
<sup>1</sup>Integrated DNA Technologies, Redwood City, CA, United States, <sup>2</sup>Integrated DNA Technologies, Coralville, IA, United States.

Introduction: Low frequency variant detection from clinically relevant samples poses significant challenges: degraded DNA from FFPE samples and low inputs from small biopsies and/or circulating cell free DNA make achieving deep coverage with high sensitivity and specificity difficult. Here we present how the use of degenerate bases during the synthesis of NGS adaptors can significantly reduce sequencing error rates, thus allowing for more accurate detection of low frequency mutations. Furthermore, we show how these molecular tagging techniques can be combined with IDT hybridization probes for extremely even and deep coverage of targeted genomic regions of interest.

**Materials and Methods:** Cell-free and FFPE DNA underwent library construction using sequencing adapters containing unique molecular barcodes. Libraries were then captured using either a pre-designed 1176kb panel or custom 82kb panel. Variants were called on samples with known mutation frequencies using MuTect v1.7.7.

**Results:** Capture with hybridization probes achieved >20% of the mean coverage for >98% of targeted bases. Mutations present at 1-5% were detected using as little as 10ng high quality DNA, 25ng FFPE DNA and <10ng cell-free DNA. Furthermore, application of error correction using molecular barcodes allowed for accurate detection of mutations present at <0.5%.

**Conclusions:** The extremely uniform target coverage achieved with IDT's pre-designed and custom enrichment panels minimizes the amount of sequence data needed to achieve deep coverage of targeted bases. When combined with the increased accuracy gained from molecular tagging strategies, researchers can achieve highly sensitive somatic variant detection even when starting with minimal amounts of clinically relevant samples.

#### P16.15

#### Development and implementation of a diagnostic pipeline for CNV calling using targeted NGS data

A. Parrish<sup>1</sup>, C. Watson<sup>2</sup>, S. Ellard<sup>1,3</sup>;

<sup>1</sup>Royal Devon & Exeter NHS Foundation Trust, Exeter, United Kingdom, <sup>2</sup>St. James's University Hospital, Leeds, United Kingdom, <sup>3</sup>Institute of Biomedical and Clinical Science, University of Exeter, Exeter, United Kingdom.

Copy number variants (CNV) are a major cause of monogenic disease, with over 30,000 CNVs reported in the DECIPHER database. A number of tools are designed to use read depth data from targeted Next Generation Sequencing (NGS) panels to identify CNVs, but in order to do so with the highest degree of sensitivity it is necessary to make modifications to account for the biases inherent in the data. Factors such as GC content and ambiguous mapping of reads due to repetitive sequence elements and pseudogenes are the principal components of technical variability. In addition, the algorithms used to interrogate the data favour detection of multi-exon CNVs, and are reliant on suitably matched normal dosage samples for comparison. We developed a strategy for calling CNVs using read depth in targeted NGS panels that divides the target intervals into 120bp windows, and utilises a pool of historic samples for comparison to overcome these limitations for use in a diagnostic laboratory. We validated our strategy against an unmodified pipeline to assess sensitivity using the R software package ExomeDepth, using a cohort of 109 samples with MLPA detected heterozygous CNVs (91 deletions, 18 duplications in 26 genes), which included 25 single exon CNVs. The unmodified strategy detected 104/109 CNVs, giving a sensitivity of 89.62% to 98.49% at the 95% confidence interval. Since our protocol detected 109/109 CNVs, we have 95% confidence that the modified strategy sensitivity is ≥96.67%, allowing the use of NGS read depth analysis for diagnostic CNV detection in a diagnostic setting.

#### P16.16

#### Search of CNVs from whole exome sequencing data and confirmation by array in patients with multiple congenital malformations and neurological disabilities

É. A. Zanardo<sup>1</sup>, A. T. Dias<sup>1</sup>, F. A. R. Madia<sup>1</sup>, A. M. Nascimento<sup>1</sup>, G. M. Novo-Filho<sup>1</sup>, M. M. Montenegro<sup>1</sup>, J. G. Damasceno<sup>1</sup>, T. V. M. M. Costa<sup>1</sup>, C. Milani<sup>1</sup>, M. Rocha<sup>1,2</sup>, F. B. Piazzon<sup>1,3</sup>, F. Monteiro<sup>3</sup>, L. S. A. Costa<sup>1,3</sup>, C. A. Kim<sup>4</sup>, F. Kok<sup>5,3</sup>, L. D. Kulikowski<sup>1,2</sup>,

<sup>1</sup>Laboratório de Citogenética, Departamento de Patologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, <sup>2</sup>Centro de Reprodução Humana e Genética, Departamento de Saúde Coletiva, Faculdade de Medicina do ABC, Santo André, Brazil, <sup>3</sup>Mendelics Análise Genômica, São Paulo, Brazil, <sup>4</sup>Unidade de Genética, Departamento de Pediatria, Instituto da Criança do HC/FMUSP, São Paulo, Brazil, <sup>5</sup>Departamento de Neurologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Detection of the copy-number variations (CNVs) is essential for investigating many genomic disorders. To date the screening by arrays allows a better detection of the CNVs making possible the conclusion of diagnosis for most patients. Recently developed, the whole exome sequencing (WES) aims to complete assessment of exonic sequences in all genes, identifying mutations in rare and genetically heterogeneous diseases. However the identification of CNVs is not used routinely in this technique and this evaluation could increase its versatility as a detection method of the variants in the genome. In this study, we evaluated 16 patients with multiple congenital malformations and neurological disabilities by WES technique for CNVs assessment. We used the array for comparison and confirmation of the results and excluding the possibility of the other CNVs. We identified several different genomic alterations in all patients by the array and/or WES, but the CNVs were confirmed in 62,5% of the patients by both techniques. Among the alterations,

deletions correspond to ~36,8% and the duplication to ~63,2%. The larger alteration found was to 10q23.12-q26.3 (12,433,500bp) in deletion and to 15q11.2-q13.3 (9,764,036bp) in duplication, and the smaller alteration found was to 9p24.2 (104,767bp) in deletion and to 12p13.31 (124,833bp) in duplication. The extraction of CNVs information obtained from the WES is an advantageous approach. Thus this study can upgrade the resolution and accuracy of search pathogenic CNVs, and improve the cost-effectiveness and reduce the number of genomic tests required to diagnosis and thus lead to a deeper understanding of disease mechanisms.

#### P16.17

#### Integrative Computational Pathogenicity Testing of GWAS Loci Asserted the important role of CCR2 gene in the Celiac Disease

N. A. Shaik<sup>1,2</sup>;

<sup>1</sup>King Abdulaziz University, Jeddah, Saudi Arabia, <sup>2</sup>Department of Genetic Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Celiac disease (CD) is a gluten intolerance disorder with underlying genetic influences. Recent fine mapping and European GWAS studies identified 57 non-HLA CD susceptible loci, majority of which are non-coding variants and with no annotated function. So, we undertook the integrative computational approach to uncover plausible mechanisms connecting these CD specific loci to the disease pathology. At first, 1,008 linked variants ( $r^2 \geq 0.8$ ) of 57 CD lead loci were ranked from deleterious to benign using CADD, GWAVA, and FATHMM algorithms. The mRNA levels of highest deleteriously ranked genes were tested in gene expression profiles of intestinal tissues and lymphocyte data. Pathway analysis was done to identify potential pathways influencing highly deleterious CD loci. We found that 2% (22 variants in 13 genes) were highly deleterious (Rank I) and 4.3% of 1065 CD variants (22 variants in 29 genes) were deleterious (Rank II). By concordant gene expression and pathway analysis we identified that deleterious SNPs localized in CCR2 gene may influence its expression and elicit cascade of immunological events associated with intestinal gluten intolerance. This study demonstrates the utility of integrative annotations, gene expression and pathway analysis for filtering the potential CD relevant variants from a large-scale genomics data.

#### P16.18

#### The linked-read exome: improved variant calls, inaccessible regions, compound heterozygotes and SV's from a single library

A. N. Fehr<sup>1</sup>, K. Giorda<sup>1</sup>, H. Ordóñez<sup>1</sup>, B. Galvin<sup>1</sup>, J. Lee<sup>1</sup>, A. Xu<sup>1</sup>, P. Marks<sup>1</sup>, I. Yousif<sup>1</sup>, E. Lin<sup>2</sup>, M. Isaaksson<sup>2</sup>, F. Useche<sup>2</sup>, K. Jeong<sup>2</sup>, M. Visitacion<sup>2</sup>, D. Roberts<sup>2</sup>, M. Coriion<sup>2</sup>, A. Vadapalli<sup>2</sup>, D. Church<sup>1</sup>;

<sup>1</sup>10x Genomics, Pleasanton, CA, United States, <sup>2</sup>Agilent Technologies, Santa Clara, CA, United States.

Exome sequencing has become a mainstay of gene-disease association in both the research and clinical settings. Despite this success, overall clinical research identifies roughly 25% of causal phenotypes, suggesting the need for improved methods. To this end, we have developed an enhanced exome that utilizes the barcoding of long DNA molecules, updated capture probes and improved informatics to produce a more complete exome assay. Of note, the new long-range information allows phasing of >90% of the genes in the exome from a single library and the addition of supplemental baits offers further increase. Additionally, the assay allows for analysis of genomic regions that are inaccessible to standard methods. For example, genes such as RPS17, which have numerous paralogs in the genome, are typically not analyzed in standard assays due to alignment ambiguity of short reads. Importantly, this approach also increases sensitivity to detecting complex variants, including CNVs and gene fusions, allowing us to phase these complex variants with SNVs. We will show examples of both germline and cancer events (copy-gain, copy-loss and copy-neutral) including a CTNS/SHPK compound heterozygote, a TMPRSS2 double-exon deletion, and an EML4-ALK fusion. The ability to detect a wide range of variant types and sizes allows for a single, integrated assay that provides a more complete view of every sample.

#### P16.19

#### Human copy number variants are enriched in regions of low-mappability

J. Monlong<sup>1,2</sup>, C. Meloche<sup>3</sup>, G. Rouleau<sup>4</sup>, P. Cossette<sup>3</sup>, S. L. Girard<sup>5,1</sup>, G. Bourque<sup>1,2</sup>;

<sup>1</sup>Department of Human Genetics, McGill University, Montreal, QC, Canada, <sup>2</sup>McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada, <sup>3</sup>Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada,

<sup>4</sup>Montreal Neurological Institute, McGill University, Montreal, QC, Canada, <sup>5</sup>Department des sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC, Canada.

Germline copy number variants (CNVs) are known to affect a large portion

on of the human genome and have been implicated in many diseases. Although whole-genome sequencing can help identify CNVs, existing analytical methods suffer from limited sensitivity and specificity. Here we show that this is in large part due to the non-uniformity of read coverage, even after intra-sample normalization, and that this is exacerbated in regions of low-mappability. To improve on this, we propose PopSV, an analytical method that uses multiple samples to control for technical variation and enables the robust detection of CNVs. We show that PopSV is able to detect up to 2.7 times more variants compared to previous methods, with an accuracy of about 90%. Applying PopSV to 640 normal and cancer whole-genome datasets, we demonstrate that CNVs affect on average 7.4 million DNA bases in each individual, a 23% increase versus previous estimates. Notably, we find that regions of low-mappability are enriched in CNVs in contrasts with somatic CNVs that are nearly uniformly distributed. We also observe that CNVs locates more than expected near centromeres and telomeres, in segmental duplications, in specific types of satellite repeats and in some of the most recent families of transposable elements. Although depleted in protein-coding genes, we identify 7206 genes with at least one exonic CNV, 324 of which harboured CNVs that would have been missed if low-mappability regions had been excluded.

#### P16.20

#### Improving detection of gene duplications in whole-genome sequencing data using allelic depth imbalance

P. Sztromwasser<sup>1,2</sup>, T. Stokowy<sup>1,2</sup>, S. Johansson<sup>1,2</sup>, V. M. Steen<sup>1,2</sup>, K. Petersen<sup>1</sup>, I. Jonassen<sup>1</sup>, <sup>1</sup>University of Bergen, Bergen, Norway, <sup>2</sup>Haukeland University Hospital, Bergen, Norway.

Copy-number variants (CNVs) are responsible for an equal share of genetic variation among humans as single-nucleotide variants (SNVs). However, detection and genotyping of CNVs using short-read sequencing is more challenging compared to calling SNVs. Four types of information are currently used to discover CNVs using whole-genome sequencing (WGS) data: (1) read depth, (2) read-pair information, (3) split reads, and (4) loss of heterozygosity (in case of deletions). Bioinformatics tools utilize these types of information, alone or in combinations, but they provide variable performance and show low concordance between the results, especially for duplications. Here we explore the utilization of an additional type of information - allelic depth imbalance (ADI) - to improve detection of duplications.

We used WGS data from a widely studied CEPH trio, and two complementary datasets of known duplications as gold-standards. The known duplications and a set of randomly selected negative control regions were ranked using ADI score. The score was based on allele ratio in heterozygous SNV calls within the duplications and control regions.

Our results show that ADI score differs between diploid and higher copy-number state regions. The score was able to classify known duplications with 67% sensitivity and 6% precision. When combined with read-depth, ADI score achieved up to 9% improvement in sensitivity and up to 7% decrease in false-positive calls, compared with read-depth alone.

Although ADI alone cannot discriminate false-positives sufficiently, used together with other signals, it can improve detection of duplications in whole-genome sequencing data

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#### P16.21

#### Cytogenomic data from a cohort of 98 brazilian patients with congenital malformations and neurological disabilities using CytoSNP-12 BeadChip Array

J. G. Damasceno<sup>1</sup>, É. A. Zanardo<sup>1</sup>, T. V. M. M. Costa<sup>1</sup>, C. Milani<sup>1</sup>, G. M. Novo-Filho<sup>1</sup>, M. M. Montenegro<sup>1</sup>, F. A. R. Madia<sup>1</sup>, R. L. Dutra<sup>1</sup>, A. T. Dias<sup>1</sup>, F. B. Piazzon<sup>1</sup>, A. M. Nascimento<sup>1</sup>, M. Rocha<sup>1,2</sup>, F. A. Marchi<sup>3</sup>, D. M. Christofolini<sup>2</sup>, A. F. L. Carvalho<sup>4</sup>, M. I. Melaragno<sup>5</sup>, C. A. Kim<sup>6</sup>, L. D. Kulikowski<sup>1,2,6</sup>;

<sup>1</sup>Laboratório de Citogenômica, Departamento de Patologia, Faculdade de Medicina da USP, São Paulo, Brazil, <sup>2</sup>Centro de Reprodução Humana e Genética da Faculdade de Medicina do ABC, Santo André, Brazil, <sup>3</sup>Laboratório NeoGene, AC Camargo Câncer Center, São Paulo, Brazil, <sup>4</sup>Departamento de Biologia Geral, Laboratório de Genética Humana e Metagênesse, Universidade Federal da Bahia, Salvador, Brazil, <sup>5</sup>Departamento de Morfologia e Genética da Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil, <sup>6</sup>Unidade de Genética, Departamento de Pediatria, Instituto da Criança, HC/FMUSP, São Paulo, Brazil.

Introduction: The advances in genomic array approaches have provided a huge improvement to detection of DNA structural variants. Here, we report on some of these variables found in patients from Cytogenomic Laboratory of FMUSP. In this study we profiled genomic alterations such as Copy Number Variations (CNVs) and Loss of Heterozygosity (LOH) in a cohort of patients with congenital anomalies and neurological disabilities that were examined beforehand with SNP-Array.

**Materials and Methods:** Ninety eight (98) patients were analyzed using cytogenomic and bioinformatics techniques in order to establish a genomic profile of Brazilian patients. All patients were submitted to the CytoSNP-12 BeadChip Array from Illumina; the data was analyzed by KaryoStudio and then post-processed with Microsoft Excel, using improved built-in functions. **Results:** The post-processed data revealed a total of 300 genomic alterations from 82 patients while 16 patients presented no alterations at all. From these 300 alterations, 55 were duplications, 113 were deletions and 132 LOHs. Chromosomes X and 22 presented the majority of structural alterations, 33 (10.5%) and 24 (7.6%) respectively and the duplications were prevalent in both chromosomes. Whereas chromosomes 21 and Y showed only a single duplication (0.32%).

**Conclusion:** With the application of arrays became possible to identify structural variants and LOH in patients with congenital anomalies and neurological disabilities that were never investigated before and also permitted an adequate genetic counseling. Our study allows drawing a novel genomic profile identifying common copy number alterations and LOH regions relevant to correlate with rare phenotypes in Brazilian patients.

#### P16.22

#### RD-Connect workshop for patient registries in Rome: "a Bring Your Own Data for beginners" on data linkage and ontologies

C. Carta<sup>1</sup>, M. Roos<sup>2</sup>, R. Kalayaperumal<sup>2</sup>, S. Gainotti<sup>1</sup>, M. Thompson<sup>2</sup>, P. Torrieri<sup>2</sup>, D. Taruscio<sup>1,2</sup>;

<sup>1</sup>Istituto Superiore di Sanità, Rome, Italy, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands.

Data on rare disease (RD) patients are collected in patient registries, biobanks and databases and represent important tools to improve diagnosis, patient management and to speed up research. However, poor interoperability and access restrictions keep data siloed.

Based on the experience from the 1st RD-Connect „Bring Your Own Data“ (BYOD), November 2014 and the results of a survey, February 2015, it was decided to organize a „BYOD for beginners“. This workshop was held in Rome on September, 2015 and was organized by Istituto Superiore di Sanità in collaboration with the RD linked data and ontologies task force, the Leiden University Medical Center, EBI, Elixir and DTL.

The workshop had as main aims: promote data sharing, show basic principles and benefits of the „data linkage approach“. Introductory lectures and a tutorial were prepared specifically for beginners. Attendees first used a traditional approach to create two mock registries and perform simple count statistics. Secondly, a „data linkage approach“ was followed using Uniform Resource Identifiers for all terms linked to ontologies to define the meaning of the terms. This facilitated merging the registries with reliable counts.

A follow up survey showed that the main lesson was clear to most participants: ontology-based data linkage makes data linkable and clearly identifiable at the source, which has the benefit of readily obtaining unambiguous answers to questions that require data from several sources.

We greatly thank participants and contributors to the workshop. Workshop was sponsored by RD-Connect and DTL/Elixir. CC, MR equally contributed to the work.

#### P16.23

#### A statistical framework for cell level predictions and eQTL deconvolution

R. Aguirre-Gamboa, N. de Klein, J. Di Tommaso, D. Zhernakova, P. Deelen, M. Bonder, Z. Borek, I. Ricaño-Ponce, BIOS Consortium, M. Swertz, I. Jonkers, S. Withoff, V. Kumar, C. Wijmenga, L. Franke, Y. Li;

University Medical Centre Groningen, Department of Genetics, Groningen, Netherlands.

We introduce a computational approach for deconvolution of expression quantitative trait loci (eQTL) effects into eQTL effects specific for cell subsets present in these complex samples. The proposed statistical framework (Decon2) consists of two components: 1) Decon-cell, that estimates cell counts using molecular profiling data (e.g. expression data or methylation results obtained from heterogeneous samples) and 2) Decon-eQTL, which subsequently deconvolutes the overall eQTL effect into cell type-specific eQTL effects.

We observed that estimated cell counts obtained with Decon-cell agree very well with the measurements determined experimentally. The correlation coefficient between predicted and measured cell/tissue proportion range from 0.75 to 0.99 using 9 different datasets. Subsequently we applied Decon-eQTL to whole blood RNA-seq and cell count data obtained from 626 individuals. We were able to replicate 40% of published neutrophil eQTLs determined using purified neutrophils [Andiappan et al. 2015]. We also redistribute the eQTLs of cis-genes in a 1Mb region around the 518 immune

disease associated SNPs from GWAS studies. Strikingly, we observed that SNPs associated with IBD (Inflammatory Bowel Disease) are over-represented in neutrophils and lymphocytes. This is in line with the recent findings [Cader et al 2013].

In summary, our method provides a universal tool which allows for redistribution of general eQTL effects of disease associated SNPs into cell type-specific eQTL effects, and this subsequently allows for implication of specific cellular subsets in a particular disease context.

#### P16.25

#### Identification of potential immune targets in controlling Endometrioid Endometrial Carcinoma metastatic progression

J. Billaud, S. Tugendreich, D. Toburen, E. Dubus;  
QIAGEN Applied Advanced Genomics, Redwood City, CA, United States.

Introduction: Endometrioid Endometrial adenocarcinoma (EEC) is a common cause of gynecological cancer death in Europe and North America. At diagnosis, 75% of women have the disease confined to the uterus, (Stage One). Five-year survival for Stage One patients is 80%, however, 15-20% develop metastasis.

Materials and Methods: Total RNA extracted from tissues obtained after surgical resection from three women at stage one EEC was subjected to RNA-sequencing. The publicly available dataset (SRP045645) was downloaded directly from the Sequence Read Archive and the FASTQ files were processed with Biomedical Genomics Workbench for secondary analysis including mapping, quantification and differential expression analysis. Through streamlined integration the data was uploaded to Ingenuity Pathway Analysis (IPA) for biological interpretation.

Results: QIAGEN Bioinformatics solutions enabled us to analyze the biological parameters involved in EEC metastatic progression from early stage in three patients diagnosed at Stage One. By comparing these patients' RNA-seq results, we determined that key Canonical Pathways and other biological processes differentiated the three patients from one other. In particular, the predicted transcriptional program allowed us to visualize key upstream drivers. Three of these transcriptional drivers are immune related molecules (two cytokines: CXCL14 and GDF15 and one growth factor FGF3). They have been predicted to be potential master regulators of a Causal Network that drives increase of EEC, metastasis, epithelial-to-mesenchymal-transition (EMT), and cellular invasion in one of the three patients.

Conclusion: Based on these results we propose these three immune molecules as possible therapeutic targets to counteract the metastatic processes in EEC.

#### P16.26

#### Epistatic SNP associations between APP and ephrin receptors APHA4 and EPHA5 in late-onset Alzheimer's disease: Enrichment of Epistatic Effects

A. Caceres, J. R. Gonzalez;  
CREAL, Barcelona, Spain.

Introduction: Amyloid toxicity remains the main hypothesis in Alzheimer's disease (AD); however, genome-wide association studies have failed to link the common genetic variability of APP (amyloid precursor protein) with late-onset Alzheimer's disease (LOAD). We hypothesized that epistatic interactions of APP with other genomic locations could mediate the link between its common variability and LOAD.

Material and Methods: We performed a novel genome-wide enrichment analysis of epistatic effects for 25 SNPs that spanned the APP genomic region. 8 APP SNPs were consistently enriched in interactions with the ephrin receptor family comprising 13 genes. We tested 36 significant interactions derived from the previous analysis in two additional validation studies.

Results: 14 interactions remained significant in the meta-analysis over the three studies (total cases/controls=3,935/2,103), confirming the epistatic enrichment of the ephrin family with APP. In particular, two interactions of APP, one with APHA4 (rs380417\*rs1025369, pvalue=0.0003) and another with APHA5 (rs2186302\*rs12499393, pvalue=0.001), were also significant in one of the validation studies. Functional data of 127 healthy brains showed 1) eQTL effects of rs380417 in both APP and APHA4 in the singular cortex (13/27 significant transcripts in APP, 10/23 in APHA4), and 2) eQTL effects of the interaction rs2186302\*rs12499393 in both APP (19/27) and APHA5 (12/23) over the whole brain.

Conclusions: Common genetic variation of APP may be associated with LOAD through interactions with EPHA4 and EPHA5; two genes previously associated with LOAD and familial AD, respectively. Transcriptomic data showed eQTL effects of numerous APP transcripts for the SNPs mediating the interactions.

#### P16.27

#### Cis-regulatory annotation of genomes in Ensembl

T. Juettemann, D. R. Zerbino, S. Wilder, A. Lyle, M. Nuhn, I. Lavidas, A. Datta, E. L. Gallego, K. Taylor, M. Ruffier, A. Yates, L. Clarke, P. Flicek;  
European Bioinformatics Institute, Hinxton, United Kingdom.

Ensembl is a leading source of information on the structure and function of the genome. The Ensembl Regulatory Build synthesises public epigenomic datasets produced by projects such as ENCODE, Roadmap Epigenomics or BLUEPRINT. We process them through a unified pipeline and make them available through a single interface. Regulatory elements annotated in isolation have limited utility; therefore we are simultaneously developing a database of cis-regulatory interactions attaching them to their target genes. Currently, two main approaches are being used to detect these interactions: genetics (e.g. eQTLs) and chromatin conformation (e.g. Hi-C). We have developed new technologies to store and display these datasets, using in particular HDF5 indexing for fast retrieval. This technology allows us to store all the GTEx summary eQTL data. Using our RESTful API, it is possible to retrieve this data simply and efficiently for any gene, variant or region, along with all other Ensembl annotations such as LD calculations from the 1000 Genomes dataset Phase 3, conservation scores, etc. It is thus possible to quickly develop advanced functional analysis pipelines without having to download or process massive data files. Finally, we are also developing high performance tools for basic research in epigenomics. The GenomeStats browser allows users to remotely compute statistics on the BLUEPRINT datasets, without downloading data or software. Thanks to the underlying WiggleTools library, it is capable of processing dozens of files simultaneously and efficiently. This means that even naïve users can try out complex hypothesis testing without getting slowed down by file management issues.

#### P16.28

#### Exome sequencing in consanguineous populations: developing a clinical diagnostic algorithm.

G. Hernández-Poveda<sup>1</sup>, A. Romera-López<sup>1</sup>, D. Cantalapiedra-De la Fuente<sup>1</sup>, V. Felipe-Ponce<sup>1</sup>, E. Mesa-Ríosquez<sup>1</sup>, Y. Moreno-Sáez<sup>1</sup>, M. L. Bellón-Robles<sup>1</sup>, C. Collado-Micó<sup>1</sup>, R. Rodríguez-De Pablos<sup>1</sup>, A. Silveyra-González<sup>1</sup>, O. Rodríguez-Cruz<sup>1</sup>, C. Buades-Gomis<sup>1</sup>, C. Casan-Martínez<sup>1</sup>, K. Al-Thihli<sup>2</sup>, D. Valero-Hervás<sup>1</sup>, S. Santillán-Garzón<sup>1</sup>,

<sup>1</sup>Sistemas Genómicos, Paterna (Valencia), Spain, <sup>2</sup>Sultan Qaboos University Hospital, Muscat, Oman.

Clinical use of whole-exome sequencing (WES), especially in patients with complex differential diagnosis and previous negative genetic tests by traditional approaches, is increasing. In certain populations, consanguinity is a frequent situation and the risk of autosomal recessive disease is directly proportional to the degree of parental relationship. In this condition, WES becomes a challenge when there is no other affected individual in the family. Developing an accurate testing strategy is necessary to reach a high diagnostic rate, avoiding wasting time and effort. We present results of a cohort of patients from unrelated consanguineous families, analysed using the Illumina HiSeq2500 platform and the SureSelectXT Human All Exon 50Mb V5 Kit (Agilent Technologies).

We obtained an average of 64,034 variants per case, which were filtered using our in-house diagnostic algorithm. We identified a homozygous disease-causing variant: c.290G>A (p.Trp97\*) in the *NDUFS4* gene, in a patient with clinical suspicion of Leigh Syndrome; and a variant of uncertain significance: c.1027G>A (p.Glu343Lys) in the *PKLR* gene in a patient with congenital hemolytic anemia on regular transfusion, providing a compatible diagnosis. Both variants were identified in large regions of homozygosity (RoH>5Mb). This points out the importance of focussing analysis on homozygous variants located in RoH in a first approach, but also looking for variants in genes implicated in the differential diagnosis and variants outside RoH in a second instance if necessary, thus establishing a useful algorithm for these families.

#### P16.29

#### GEUVADIS European Exome Variant Server: Variant allele frequency aggregation from multicentre, access-restricted data

M. Bosio<sup>1</sup>, O. Drechsel<sup>1,2</sup>, R. Rahman<sup>1,2</sup>, T. Wieland<sup>3</sup>, G. Bertier<sup>3,4</sup>, C. Gillissen<sup>7</sup>, L. Domenech Salgado<sup>1,8</sup>, A. Prasad<sup>1</sup>, K. Rabionet<sup>1</sup>, S. Laurie<sup>9,8</sup>, S. Beltran<sup>9,8</sup>, T. Spector<sup>10</sup>, J. Veltman<sup>7,11</sup>, T. Strom<sup>12,4</sup>, X. Estivill<sup>1,8</sup>, S. Ossowski<sup>1,8</sup>,

<sup>1</sup>Centre for Genomic Regulation CRG, The Barcelona Institute of Science and Technology, Barcelona, Spain, <sup>2</sup>Institute of Molecular Biology (IMB), Ackermannweg 4, 55128, Mainz, Germany, <sup>3</sup>The Netherlands Cancer Institute NKI, Amsterdam, Netherlands,

<sup>4</sup>Institute of Human Genetics, Technische Universität München, 81675, Munich, Germany,

<sup>5</sup>Centre of Genomics and Policy - McGill University 740 Dr. Penfield Avenue, Montreal, QC, Canada, <sup>6</sup>UMR 1027, Inserm, Université Toulouse III - Paul Sabatier 37 allées Jules Guesde F-31000, Toulouse, France, <sup>7</sup>Department of Human Genetics, Radboud University Medical Center, 6525 GA, Nijmegen, Netherlands, <sup>8</sup>Universitat Pompeu Fabra (UPF),

Barcelona, Spain, <sup>9</sup>CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Baldíri i Reixac 4, 08028, Barcelona, Spain, <sup>10</sup>Genetic Epidemiology, Twin Research and Genetic Epidemiology, King's College, London, United Kingdom, <sup>11</sup>Department of Clinical Genetics, Maastricht University Medical Centre, 6229 HZ, Maastricht, Netherlands, <sup>12</sup>German Research Center for Environmental Health, 85764, Neuherberg, Germany.

**Introduction:** Reliable tools discerning between rare and common genetic polymorphisms within a local context are essential for effective analysis of NGS clinical data for disease studies. Initiatives like 1000Genomes and ExAC have released population allele frequencies (AF) for single nucleotide polymorphisms (SNPs) and insertion-deletions polymorphisms (INDELS). Albeit useful, they consider Europe as an entity, are biased towards a few European subpopulations, and require centralised raw data processing.

**Materials and Methods:** GEEVS (GEUVADIS European Exome Variant Server) integrates multi-centre restricted-access AF data, and offers country-specific variant characterisation. Contributing centres apply standardised GEEVS protocols for variant calling (integrating several tools and custom filters), quality control, and variant aggregation. Only aggregated AF and coverage profile data are shared with the central server. The "master aggregator" function performed at the central server subsequently produces European and country specific AFs, and enriches each variant with functional information, damage predictions (SIFT, PolyPhen2), and OMICs information (dbSNP, 1000Genomes, EVS, UCSC Genome Browser).

**Results:** GEEVS aggregates variants from 2817 individuals of European ancestry, by integrating anonymised, restricted-access data from five European contributors. GEEVS checks data quality in terms of AF distribution, Ti/Tv ratio, AF concordance with 1000Genomes, and haplotype concordance with Genome In A Bottle gold standard. GEEVS achieved quality values similar to ExAC without requiring centralised processing.

**Conclusions:** GEEVS extracts high-quality aggregated AFs from restricted-access clinical NGS data and computes country-wise variant characterisations, providing precise local-context information for disease studies. GEEVS, available from <http://geevs.crg.eu/>, or <http://www.ebi.ac.uk/eva> (European Variation Archive), accepts new submissions of European aggregated AF data.

## P16.30

### The Human Early-life Exposome (HELIX) project: exposome, molecular mechanisms and child health

**M. Vives Usano**<sup>1,2</sup>, **M. Bustamante**<sup>1,2,3</sup>, **O. Robinson**<sup>2,3,4</sup>, **C. Hernández**<sup>2,3,4</sup>, **Y. Sarria**<sup>1</sup>, **J. González**<sup>2,3,4</sup>, **Á. Carracedo**<sup>5,6,7</sup>, **E. Sabido**<sup>1,2</sup>, **M. Coen**<sup>8</sup>, **M. Vrijheid**<sup>2,3,4</sup>, **X. Estivill**<sup>1,2,4</sup>

<sup>1</sup>Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain, <sup>4</sup>CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain, <sup>5</sup>Grupo de Medicina Xénómica – Universidad de Santiago de Compostela, Santiago de Compostela, Spain, <sup>6</sup>CIBER Enfermedades Raras (CIBERER), Madrid, Spain, <sup>7</sup>Fundación Pública Galega de Medicina Xénómica (SERGAS), Santiago de Compostela, Spain, <sup>8</sup>Biomolecular Medicine, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College London, London, United Kingdom.

The exposome is defined as the totality of human environmental exposures from conception onwards, and it complements the genome. Thus, it might explain part of the variation in complex phenotypes.

The European FP-7HELIX project (The Human Early-life Exposome) aims to elucidate the effect of the exposome on child health (growth and obesity, lung health, and neurodevelopment), and to understand the molecular mechanisms underlying these associations by the use of new multiple's omics technologies.

Over 1,200 children aged 6-9 years from six existing birth cohorts across Europe participate in the study. The exposome was assessed through multiple biomarkers of exposures (e.g. metals and organic pollutants), questionnaire (e.g. diet), geographic information systems (e.g. air pollution), and personal monitors (e.g. physical activity).

In the same children, the metabolome, proteome, transcriptome and epigenome were analyzed. Methylation of DNA in leukocytes was analyzed with the Human Methylation 450 BeadChip array. Whole blood gene expression was quantified using the Affymetrix HTA 2.0 array and more than 2,000 miRNAs were measured using the Agilent SurePrint Human miRNA Microarray rel 21. Untargeted nuclear magnetic resonance spectroscopy (NMR) and targeted profiling with the BiocratesAbsoluteIDQ p180 assay kit were used to analyze metabolites in urine and serum, respectively. Finally, a set of proteins related to inflammatory and metabolic signaling were analyzed using the antibody-based multiplex platform Luminex. Genome-wide genotyping data is available for one third of the children.

This unique molecular dataset will help to elucidate the molecular mechanism through which the exposome affects health.

## P16.31

### Prediction of genome-wide SNPs effects for testis weight in a house mouse

**B. Karacaören;**

*Akdeniz University, Antalya, Turkey.*

**Introduction:** Genome-wide association studies (GWASs) used to detect candidate genes for complex traits. However assumptions regarding underlying genetic architecture of the traits are still under discussion referring the „missing heritability“ problem. For instance one may assume either major genes or numerous small genes that might control trait of interest. The objective of this study was to prediction of SNPs effects (by assuming both major gene and polygenic inheritance simultaneously) using testis weight of house mouse to detect fertility related loci.

**Material and Methods:** We used 185 house mouse (Turner and Harr (2014)) with 156202 SNPs for mapping variants for relative testis weight. We assumed mixture of different normal distributions for the SNP effects to be predicted. We also used single SNP model to evaluate only major gene assumption for fertility trait.

**Results:** Single SNP approach detected genomic signals from 8 loci (chromosomes of 1, 6, 12, 14, 17, X). However after multiple hypothesis testing correction we could only confirm suggestive genomic signals using single SNPs approach. When we assumed polygenic inheritance 1123 SNPs explained all of genomic variance. Most of those SNPs (>0.98 of them) had tiny effect. JAX00486833 at chromosome 2 had the highest explanatory variance (0.057).

**Conclusion:** These findings show that assuming different effect sizes for the SNPs lead to better genomic predictions for testis weight. This stresses the importance of the assumptions regarding genetic architecture of the complex traits used in GWAS.

The study was supported by Akdeniz University Scientific Research Projects Unit, project number: FBA-2015-1117.

## P16.32

### Gene expression data signal correction to identify disease gene signals

**S. van Dam;**

*UMCG, Groningen, Netherlands.*

Disease phenotypes manifest both at physical and genetic level. The alterations in the physical state of a patient can help to identify its underlying cause such as the presence of a genetic abnormality or an infection. However, often it is not possible to identify these abnormalities, underlying a phenotype or only on a broad level, using the limited number of variables derived from the physical state of a patient. Using gene expression profiles as a marker for disease, the expression of each gene can be used as a variable greatly extending the number of measurable variants. We have previously shown that it is possible to identify over expressed genetic regions in cancer patients, which proved to correlate with genomic duplications. These could potentially be used as biomarkers for different cancer types. In this project we aim to extend this analysis to other phenotypes using RNA-seq data, which is more sensitive. Using RNA-seq blood data from over 5,000 extensively phenotyped patients, we aim to identify gene expression variation that is unique to patients that share specific phenotypes, such as obesity, smoking and depression. To achieve this, we correct samples from patients harnessing the phenotype with signals that occur in individuals that do not have this phenotype. The residual signal then represents/explains the abnormal genetic phenotype. These signals can be investigated to identify key players underlying the phenotype and potentially be used as biomarkers for diagnosis that potentially allow to differentiate between different sub-types of disease.

## P16.33

### A novel network method (digNet) identifies a densely interconnected gene network associated with childhood-onset asthma

**Y. Liu**<sup>1,2</sup>, **D. Roqueiro**<sup>3</sup>, **M. Brossard**<sup>1,2</sup>, **P. Margaritte-Jeannin**<sup>1,2</sup>, **M. H. Dizier**<sup>1,2</sup>, **E. Bouzigon**<sup>1,2</sup>, **F. Demenais**<sup>1,2</sup>, *The Gabriel Asthma Consortium*

<sup>1</sup>INSERM UMR-946, Paris, France, <sup>2</sup>Université Paris Diderot, Paris, France, <sup>3</sup>Machine Learning and Computational Biology Lab, Department of Biosystems Science and Engineering, ETH, Basel, Switzerland.

**Introduction:** Besides single marker-based analysis classically used in genome-wide association studies (GWAS), network-based approaches have become promising to identify the joint effect of multiple genetic factors. Most of network-based methods proposed to date aim at finding genetic units that are connected in a biological network, no matter the strength of connections. This can be problematic since loose connections are non-robust to

noise from either the GWAS signals or the biological resource used to build the network.

**Methods:** We propose digNet, a novel network-based method integrating GWAS outcomes and protein interaction network (PIN) to identify a densely interconnected gene sub-network enriched in association signals. Our method is based on zero-one quadratic optimization, which can be solved exactly and efficiently through min-cut algorithms. The digNet approach possesses two desirable properties: (i) the selected genes are densely interconnected, thus functionally related; (ii) the results are robust against noise. **Results:** We applied digNet to the outcomes of two meta-analyses of 9 childhood-asthma GWASs each (~3000 cases/3000 controls in each meta-analysis). We identified a gene sub-network of 172 genes that was consistent across the 2 datasets. This network is significantly associated with childhood asthma ( $P<10^{-5}$ ) and shows strong interconnection ( $P=7\times 10^{-5}$ ). Out of the 172 genes, 5 are known asthma genes while 148 genes, with nominally significant gene-wise P-values, represent novel candidates. Nine immune-related gene families and KEGG pathways were found enriched in these genes. These results show the advantage of our method to identify novel functionally related candidate genes for asthma.

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## P16.34

### Independent components of gene expression in endurance runners

*M. Maqueda<sup>1,2</sup>, E. Roca<sup>3</sup>, J. M. Soria<sup>4</sup>, A. Perera<sup>1,2</sup>;*

<sup>1</sup>Universitat Politècnica de Catalunya, Barcelona, Spain, <sup>2</sup>Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine, Barcelona, Spain, <sup>3</sup>Universitat de Girona, Girona, Spain, <sup>4</sup>Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

**Introduction:** This study assessed the gene expression (GE) response from a group of 16 endurance runners after participating in an 82km competition. **Material and Methods:** We used microarray technology (HuGene2.0st from Affymetrix, Inc., California) to analyse the genome-wide GE profile of runners' blood samples that were collected before and after the race. A linear regression model was fit to each transcript cluster (TC) expression value considering gender and completed distance as covariates. Statistically significant differentially expressed TCs were obtained from a moderated t-statistics performed for each coefficient in the model (adjusted p-value 5%, FDR). An over-representation analysis was applied to the differential TCs querying KEGG PATHWAY and The Gene Ontology (GO) databases. An independent component analysis (ICA) was computed to extract the independent expression modes among the differential TCs.

**Results:** 5084 distinct genes were prioritized being 37% down-regulated and 63% up-regulated. Over-represented biological pathways were mostly associated with: genetic information processing, infectious disease and immune system. ICA identified seven independent regulation expression modes as response to exercise.

**Conclusions:** The intervention impacted heavily on the gene regulation processes, so that up to one fourth of the total variance related to coding RNA activity was captured as response to the exercise. This response produced extensive alterations in several pathways in the human biology, which we decomposed through a statistical method targeting independent expression modes.

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## P16.35

### TumOnc: differential algorithm of identification of driver mutations in oncogenes and tumor suppressor genes

*F. Bezrukov, S. Nikolaev, S. E. Antonarakis;*

*University of Geneva, Geneva, Switzerland.*

Cancer is a genetic disorder associated with high rates of accumulation of somatic mutations. However the majority of mutations are neutral and have no impact on the cell, and only few are under positive selection and are observed in cancer more frequently than expected by chance.

Several statistical algorithms exist to detect putative cancer driver genes, however they do not exploit the mutational properties of oncogenes and tumor suppressors.

TumOnc, the novel algorithm for detection of driver mutations in cancer estimates the non-uniform genomic background mutation rates as a function of tumor type specific mutational profile in three-nucleotide context, DNA replication timing, chromatin state and transcriptional activity. The principal novelty of the TumOnc algorithm is based on the assumption that the oncogene may contain a very limited number of sites which upon gain-

of-function mutation turn proto-oncogene to oncogene and subsequently are characterized by recurrent mutations.

In combination with the calculated background mutation rates per nucleotide and per patient this allowed us to identify significantly recurrent mutations, with sensitivity for oncogenes superseding existing algorithms. TumOnc is also designed for the detection of tumor suppressor genes searching for loss-of-function variants in both alleles. TumOnc has been recently tested in Basal Cell Carcinoma which has very high mutation rates (65M/Mb) and its usage has identified novel driver mutations (Bonilla et al., *Nature Genetics* in press).

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## P16.36

### GWAS2Target: A gene prioritization approach to identify new therapeutic concepts exemplified for Schizophrenia

*E. Simon;*

*Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany.*

The identification new therapeutic concepts for human diseases is challenging because it requires a deep understanding of the molecular pathology. Genome wide association studies (GWAS) have become a powerful tool to disclose new molecular mechanisms of complex diseases. However, the development of new therapies based on these findings is hampered by additional hurdles i.e. causal gene mapping and target efficacy & feasibility.

We have developed a new gene prioritization approach using weighted scores for target gene feasibility, function, specificity & novelty. Each score is derived from multiple sub-scores which again can be weighted according to specific requirements. We applied this approach to a recent GWAS for Schizophrenia (SZ) including 108 loci.

Causal gene mapping has been parameterized by using the number of coding genes per loci as an inverse estimate for target feasibility. As additional sub-score for this dimension we applied the gene protein product class and the homology to model organisms. Gene function has been parameterized by GWAS significance & literature evidence. Target specificity has been derived from genome wide expression datasets in different sub-categories (brain vs. periphery, cell type specific and pattern in cortical sub-regions). Target novelty has been estimated from the inverse number of SZ related publications.

The combined scores with flexible weighting allows interactive ranking of the 340 coding genes. Using default weight parameters predicts some well-known SZ target genes at top ranks i.e. CACNB2 (#1), DRD2 (#2) or GRM3 (#10). In the near future, we plan to integrate additional datasets for gene mapping and function.

## P16.37

### A high-performance and scalable genomics platform for variation exploration using clinical traits

*M. Haimel<sup>1</sup>, J. Coll<sup>2</sup>, A. Rendon<sup>2</sup>, N. Morrell<sup>1</sup>, S. Gräf<sup>2,3</sup>, I. Medina<sup>4</sup>;*

<sup>1</sup>Department of Medicine, University of Cambridge, Cambridge, United Kingdom,

<sup>2</sup>Genomics England, London, United Kingdom, <sup>3</sup>Department of Haematology, University of Cambridge, Cambridge, United Kingdom, <sup>4</sup>High Performance Computing Service, University of Cambridge, Cambridge, United Kingdom.

Large-scale genomic studies are generating trillions of variant calls and tens of terabytes of data per day. The integration of this data with other heterogeneous information adds to the challenges in data processing. An integrated analysis of high-throughput whole genome sequencing data of tens-of-thousands of individuals requires a scalable system that enables the continuous addition, normalization, merging and annotation of variants.

Apache Hadoop is a scalable framework for storage and large-scale, distributed processing of petabytes of data. HBase, an add-on module based on Hadoop, enables storing and querying large amounts of heterogeneous data returning results in a matter of milliseconds for basic queries and just a few seconds for more complex queries.

We present a software platform that is designed to continuously normalize, transform and load gVCF files into HBase, as well as provide up-to-date variant annotations and allele counts for large cohorts. Interactive response times allow user authenticated genome exploration through a web browser, command line or API for custom analysis provided by the open-source OpenCGA platform.

Here we demonstrate the performance of our implementation of this platform using data generated from more than 5,000 whole genomes sequenced by the NIHR BioResource Rare Diseases Study, coupled with phenotypic information. Integration of other 'omics' data is ongoing to facilitate the discovery of novel pathogenic variants even beyond the protein-coding space.

The development of interactive tools for data exploration is now feasible due to quick response times and the ability to undertake complex queries across the Hadoop infrastructure.

## P16.38

### Variant calling performance of a next generation sequencing panel for single nucleotide and indel variants in hereditary spastic paraplegias

**P. Cacheiro<sup>1</sup>, A. Ordóñez-Ugalde<sup>1</sup>, B. Quintáns<sup>2</sup>, J. Amigo<sup>2</sup>, Á. Carracedo<sup>1,2</sup>, M. J. Sobrido<sup>2</sup>,**  
<sup>1</sup>Grupo de Medicina Xenómica, CIBERER, CIMUS, Universidad de Santiago de Compostela, Santiago de Compostela, Spain, <sup>2</sup>Fundación Pública Galega de Medicina Xenómica, Instituto de Investigación Sanitaria de Santiago de Compostela, Santiago de Compostela, Spain.

**Introduction:** Hereditary spastic paraplegias (HSP) are genetically heterogeneous neurodegenerative disorders expected to benefit from next-generation sequencing (NGS) technologies. However, the optimal parameters for variant detection and filtering still need careful evaluation in order to standardize these techniques for clinical applications. In this study we analysed the performance of several variant calling programs on an HSP gene panel. **Materials and Methods:** The dataset derives from a targeted panel of 30 HSP genes sequenced in 83 samples, in which 187 SNPs and indels (56 unique) were also detected by Sanger sequencing. The variant calling efficiency of 3 algorithms (Lifescope, GATK UnifiedGenotyper and GATK HaplotypeCaller) was examined. Genotyping quality, depth of coverage and percentage of variant allele were considered to assess the quality of the calls.

**Results:** Discrepancies between NGS results and those from Sanger were found for 7 variants. Two SNPs were not detected by any of the variant calling algorithms because the individuals were homozygous for the rare allele but concordant with the reference sequence. One SNP in a low coverage region (6X) was only detected by two algorithms. Four indels were called differently by each of the callers, with ambiguities regarding annotation and zygosity.

**Conclusions:** In NGS protocols the calling efficiency may differ among callers, influenced by variant type and coverage. Variant detection protocols need to account for the presence of rare variants in the reference sequence and for ambiguities in indel calling. Threshold for quality scores should be cautiously established.

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## P16.39

### Human Knockout in Italian isolated population

**C. Barbieri<sup>1</sup>, M. Mezzavilla<sup>2</sup>, M. Cocca<sup>3</sup>, C. F. Sala<sup>1</sup>, P. Gasparini<sup>2,3,4</sup>, N. Soranzo<sup>5,6</sup>, D. Toniolo<sup>1</sup>,**

<sup>1</sup>San Raffaele Scientific Institute, Division of Genetics and Cell biology, Genetics of common disorders Unit, Milan, Italy, <sup>2</sup>Division of Experimental Genetics, Sidra Medical and Research Center, Doha, Qatar, <sup>3</sup>Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy, <sup>4</sup>Medical Genetics, Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy, <sup>5</sup>The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1HH, Cambridge, United Kingdom, <sup>6</sup>Department of Haematology, University of Cambridge, Long Road, Cambridge CB2 0PT, United Kingdom.

Null mutations in homozygous state could generate 'human knockouts' for a gene. The study of these variants can provide insight into gene function. Our approach was focused on Italian isolated populations, from North (Friuli Venezia Giulia and Val Borbera) and South (Carlantino), to find out natural gene knockouts.

926 individuals were sampled and sequenced from nine different isolated villages, resulting in a catalogue of up to 19 millions variants (43% common between all villages) with 41% to 50% private variants at lower frequencies (MAF < 1%). Loss of Function (LOF) variants were investigated in the following functional categories: frameshift, stop lost, initiator codon, splice acceptor, splice donor, stop gained. Only variants with at least 1 individual homozygote for the alternative allele were selected and a set of ~2300 null mutation was created.

Among these: 90% was found in 1000 Genome Phase III panel and the remaining 10% was novel.

The site-frequency spectrum for these null mutations showed that 0.3% of the total is rare (AF<0.05) and about the 30% is common (AF>0.4).

Our analysis revealed the presence of up to 2200 genes in knock out state, the majority shared between the three isolated populations, while fewer genes are specific of each population.

Preliminary analysis of frameshift variants shows that in many instances only few of the alternative transcripts in a gene appear interrupted, suggesting a limited effect for most LOFs.

Our findings in combination with phenotypic data available in our cohorts will provide a focal point into understanding specific gene functions.

## P16.40

### Improved in silico protein predictions for missense variants in genes involved in congenital hypogonadotropic hypogonadism

**D. Cassatella<sup>1,2</sup>, J. Acierno<sup>2</sup>, C. Xu<sup>1,2</sup>, B. Stevenson<sup>3</sup>, N. Pitteloud<sup>1,2</sup>,**

<sup>1</sup>University of Lausanne, Lausanne, Switzerland, <sup>2</sup>Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, <sup>3</sup>Vital-IT, Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder characterized by absent or incomplete puberty, and infertility due to a lack of GnRH secretion. CHH is a rare disorder with more than 30 genes known to underlie the phenotype. These genes code for a heterogeneous group of proteins with a significant proportion of reported mutations being missense changes. This underscores the importance of prediction algorithms in determining whether these variants are pathogenic or benign.

To assess the accuracy of the available in silico prediction tools, published pathogenic (n=158) and benign (n=27) missense variants from the ClinVar database and our own functionally-validated mutations in CHH known genes were evaluated. dbNSFP was queried to annotate functional predictions from 12 algorithms: SIFT, PolyPhen2\_HDIV, PolyPhen2\_HVAR, LRT, MutationTaster, MutationAssessor, FATHMM, PROVEAN, VEST, CADD, MetaSVM and GERP++.

ROC curves, area under the curve (AUC) and Matthews Correlation Coefficient (MCC) calculations showed that the most accurate predictors were VEST (AUC=0.87, MCC=0.49), PolyPhen2\_HDIV (AUC=0.84, MCC=0.45) and CADD (AUC=0.81, MCC=0.42). Pairwise analysis of all prediction tools demonstrated improved performance relative to individual tools, with the top three combinations being VEST+PolyPhen2\_HDIV and VEST+CADD (both, MCC=0.54, accuracy=89.2%, sensitivity=94.9%, specificity=55.6%), followed by PolyPhen2\_HDIV+CADD (MCC=0.53, accuracy=85.4%, sensitivity=87.3%, specificity=74.1%).

Given these results, the combination of PolyPhen2\_HDIV+CADD was chosen as it provides an improved balance between accuracy, sensitivity, and specificity. Future studies will evaluate larger datasets of mutations, with more balanced numbers of pathogenic and benign changes, to validate this finding of improved tool combinations to assess clinically relevant missense variants in CHH genes.

## P16.41

### Computational analysis on Pathogenic mutations of Human Interlukin Receptor Alpha Gene

**B. Banaganapalli;**

*King Abdulaziz University, Jeddah, Saudi Arabia.*

**Introduction:** Interlukin 10 receptor alpha (IL10RA), is a one of the key partner in inflammatory pathway, recent advent of GWAS studies and - exome studies has started to uncover known/novel pathogenic variations in IL10RA gene in several autoimmune diseases. However, the structural and functional impact of these genetic mutations is not well identified. Hence, this study has performed the computational analysis of clinically potential missense and untranslated region mutations of human IL10RA gene.

**Materials and Methods:** In this study a combination of empirical rule and support vector machine based insilico algorithms were employed to predict the pathogenic potential of non-synonymous mutations of 1L10 gene. Additionally, molecular modeling and secondary structure analysis was performed to confirm their impact on the stability and secondary properties of IL10RA protein.

**Results:** Besides, the mutations corresponding to p.Y57C, p.T84I, p.Y91C, p.R101W, p.R117C, p.R117H and p.G141R in exonic region; c.\*1537T>C, a regulatory region variant was also found to potentially influence the structural and functional deviations of IL10RA activity. Moreover, the molecular docking analysis of IL10RA with substrate (IL10) and mutated forms of IL10RA was found to be three mutated proteins of IL10RA are (p.Y57C, p.R117C and p.G141R) most interesting residues for mutagenesis and affect the selectivity and affinity of IL10RA towards IL10.

**Conclusion:** Our findings are expected to help in narrowing down the number of IL10RA genetic variants to be screened for auto immune disease association studies and also to design a better competitive inhibitors for mutated forms IL10RA protein.

## P16.42

### Genetical genomics of hepatic transcriptome in recombinant inbred rat models of metabolic syndrome

**B. Chylikova, M. Hodulova, M. Krupkova, L. Šedova, D. Křenova, V. Křen, O. Šeda;**

*Institute of Biology and Medical Genetics First Faculty of Medicine Charles University in Prague, Prague, Czech Republic.*

The genetically designed set of recombinant inbred rat strains PXO serves as a model of human metabolic syndrome. Aim of this study was to analyse the genomic architecture of the metabolic syndrome features in this model set using an integrated genomic, phenomic and transcriptomic approach. We have accumulated over 100 directly measured metabolic, morphometric and hemodynamic parameters in the complete set of 16 PXO strains and their progenitors, SHR-Lx and BXH 2 rat strains. Total RNA was isolated from liver of adult males of all strains, its integrity was checked by Agilent 2000 BioAnalyzer. The transcriptomic assays were run using Affymetrix® Rat Gene 2.1 ST Array Strip. Using the genomic information of > 20,000 SNPs, we have performed a genetical genomic study and correlated the expression profiles with the phenomic dataset. Resulting data were subjected to systems biology-level analyses using Partek Genomics Suite and Ingenuity Pathways Analysis.

We have identified over 55 transcripts and their respective cis- and trans-eQTLs significantly (FDR < 0.05) distinguishing the PXO strains and at the same time correlated to determinants of metabolic syndrome (including Echdc2, Tmem14c, Miox and Rarres2). Integrative analysis revealed several networks likely to drive the main differences in metabolic syndrome aspects in the PXO set with Il1b, Sirt2, Ppara, Ppargc1b, Atp7b genes as their major nodes.

Using integrative approach, we have identified major biological networks contributing to the pathophysiology of several aspects of metabolic syndrome in the recombinant inbred model set.

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#### P16.43

#### Deconvolution of cancer cell hierarchies through large-scale analysis of gene expression

*L. Järvinen, U. Gullberg, B. Nilsson;  
Lund University, Lund, Sweden.*

**Introduction:** Increasing evidence supports that some solid tumors and leukemias are organized as cellular hierarchies, sustained by cancer stem cells. Molecular markers for different stages in these hierarchies could potentially be exploited for targeted therapies.

**Materials and Methods:** Here we introduce a novel, computational approach to characterize tumor cell hierarchies. In sharp contrast to traditional approaches, which rely on physical cell sorting and analysis of sorted cell fractions, we exploit high-dimensional matrix decomposition to identify genes that discriminate the different stages in cancer cell hierarchies through deconvolution of gene expression profiles of unsorted tumor cells. Importantly, our approach completely avoids the need for physical cell sorting. Instead, genes that discriminate different stages in the neoplastic hierarchy are identified by mathematical unmixing. To obtain robust results, the process is constrained by L1 regularization and a priori information about normal cell development in the same tissue.

**Results:** In systematic validation experiments, we demonstrate that our approach is capable of recovering cell type-specific markers, and cell frequencies, from real and simulated expression profiles of mixed cell populations. Particularly, using large-scale unmixing of unsorted Acute Myeloid Leukemia samples, we were able to identify both known and new markers for leukemic stem cells.

**Conclusion:** Our work establishes deconvolution of expression profiles of mixed tumor cell populations as an innovative approach to characterize tumor cell hierarchies, potentially facilitating the development of therapies towards cancer stem cells.

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#### P16.44

#### Assessing tumour heterogeneity and evolution in non-Hodgkin lymphoma (NHL) using liquid biopsies and single cell sequencing

*R.D. Morin<sup>1</sup>, S. Yu<sup>1</sup>, M. Alcaide<sup>1</sup>, B. Grande<sup>1</sup>, S. Assouline<sup>2</sup>, K. Mann<sup>2</sup>, N. Johnson<sup>2</sup>;  
<sup>1</sup>Simon Fraser University, Burnaby, BC, Canada, <sup>2</sup>Jewish General Hospital, Montreal, QC, Canada.*

**Introduction:** Apoptotic tumour cells shed DNA that ultimately becomes circulating tumour DNA (ctDNA), which can be recognized in plasma by the presence of tumour-specific alterations. Dynamics in the level of ctDNA can reflect changes in tumour burden within a patient and, as such, its measurement is a highly specific and potentially versatile biomarker for solid cancers.

**Materials and Methods:** Various methods for quantifying ctDNA exist including those relying on massively parallel sequencing. We use these to quan-

tify ctDNA in patients with NHLs and evaluate its utility as a biomarker and with molecular tagging strategies, are exploring ctDNA as a substrate for non-invasive genetic characterization. Computational approaches facilitate inference of clonal populations within tumours populations and we used these to determine whether ctDNA recapitulates this clonal structure.

**Results:** We have detected ctDNA in most relapsed NHL patients with many cases exhibiting levels that facilitate genetic characterization. We show high specificity for ctDNA changes in predicting treatment response. Our methods enable non-invasive characterization of tumour heterogeneity including mutations in plasma that were poorly supported in the matched biopsy. Sequencing of serial plasma samples has also revealed clonal evolution including the emergence of mutations in genes that likely contributed to treatment resistance including STAT6, MS4A1 and NR3C1. We used single cell sequencing methods to confirm and refine clonal populations in NHLs allowing resolution of evolutionary history of treatment-resistant tumours. **Conclusion:** Sequencing of ctDNA is a powerful means to study somatic tumour genetics non-invasively and can inform on clonal complexity and evolution in NHLs.

#### P16.45

#### Assessing the Ion Torrent technology for its use in human lung microbiome profiling with 16S rRNA amplicon sequencing

*B. Guillen-Guio<sup>1</sup>, A. Espuela-Ortiz<sup>2</sup>, F. Lorenzo-Diaz<sup>2,3</sup>, A. Baez-Ortega<sup>4</sup>, J. Alcoba<sup>5</sup>, T. E. Klassert<sup>6</sup>, H. Slevogt<sup>6</sup>, M. Pino-Yanes<sup>1,7</sup>, C. Flores<sup>1,2,7</sup>;*

<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain, <sup>2</sup>Applied Genomics Group (G2A), Section of Genetics, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, Tenerife, Spain, <sup>3</sup>Genetics Laboratory, Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Universidad de La Laguna, Tenerife, Spain, <sup>4</sup>Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, <sup>5</sup>Microbiology Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain, <sup>6</sup>Sepomics Research Centre, Jena University Hospital, Jena, Germany, <sup>7</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

**Introduction:** Sepsis is a major cause of death in the adult intensive care units worldwide. Lung microbiome shifts may associate with severity in sepsis. Here we assessed the performance of the Ion Torrent sequencing platform for microbiome profiling.

**Materials and Methods:** We used the Ion Torrent PGM (Thermo Fisher) for 16S rRNA V4 amplicon-based sequencing. Performance was assessed by using pooled genomic DNA from 20 bacteria compared to that of a MiSeq instrument (Illumina). Then, a mixture of 12 clinical bacterial strains commonly isolated from lungs was used to compare the performance of distinct DNA extraction procedures. Pre-processing, taxonomic classification, and diversity analyses were done with a QIIME-based pipeline.

**Results and Conclusions:** While virtually all reads were full-length for MiSeq, these constituted only 33.4% of the reads for Ion Torrent. We found good agreement between the relative bacterial abundance at the genus level between the two platforms only if fragment size filtering was relaxed in the Ion Torrent reads. Furthermore, the use of DNA extraction methods combining chemical and mechanical lysis significantly improved diversity and abundance estimates, allowing to better recapitulate the bacterial community. These results have major practical implications for the following studies being conducted in specimens of human origin.

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#### P16.46

#### GIML: greedy inference of methylation levels

*E. Raineri;  
CNAG, Barcelona, Spain.*

Changes in methylation along the DNA are often revelatory of what regulatory activities are taking place in a cell. Such variations have been observed in promoter regions, along gene bodies, in enhancers, at splicing sites and elsewhere. Whole genome bisulfite sequencing can measure cytosine methylation with 1bp resolution over an entire genome; the interpretation of these data needs analysis tools which discern noise from relevant structures. Here I explain a simple greedy algorithm designed to analyze methylation by first segmenting it into homogeneous regions. The definition of homogeneous can be tuned to the problem at hand, given that this procedure can find both many small (almost constant) stretches or fewer large blocks which contain more substantial variation. The package (called GIML) analyzes an entire genome at ~30000 positions/sec.

I ran the algorithm on data generated by the Blueprint consortium looking for positions where one region of very low methylation is consistently surrounded by stretches of very high methylation : these „jumps“ are enriched in transcription start sites. GIML can also find differentially methylated regions with different lengths across samples.

Multiscale smoothing algorithms play an essential role in unraveling epigenetic measurements, by making visualization easier and distinguishing experimental artifacts from the underlying biological signal. The long term question is how do these experimental data correlate with each other and what do they tell us about molecular mechanisms: I think that the kind of quick, principled simplification of methylation data performed by GIML is an useful step in answering that question.

#### P16.47

#### Estimating interaction of host genetics to the microbiome structure on the large Dutch population cohort

A. Kurilshikov<sup>1,2</sup>, M. J. Bonder<sup>1</sup>, P. Deelen<sup>1</sup>, E. Tigchelaar<sup>1</sup>, L. Franke<sup>1</sup>, C. Wijmenga<sup>1</sup>, J. Fu<sup>1</sup>, A. Zhernakova<sup>1</sup>;

<sup>1</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>2</sup>Novosibirsk State University, Novosibirsk, Russian Federation.

**Introduction:** The gut microbiome inhabiting human intestine plays important role in the processes occurred in human body. Many environmental and intrinsic factors are linked with gut microbiome, with both one-way causations (age, gender) and reciprocal relations (BMI, blood lipid level, medication). It was shown earlier that some bacteria inhabiting human gut are heritable, so the human genome can influence the microbiome. In this work, we performed statistical analysis to estimate the effect of SNPs to the microbiome structure.

**Materials and methods:** The data from 981 individuals from the LifeLines-DEEP population cohort was used. For each individual, both genotyping (Immunochip, Illumina) and metagenomic sequencing (paired-end Illumina reads) were performed. The preprocessing of the microbiome data included QC control and filtering from human DNA followed by MetaPhlAn2.2 mapping. Multiple distance matrices (Bray-Curtis, Jaccard, weighted/unweighted UniFrac) were used as a dependent variable to estimate the SNP effect on microbial structure.

**Results:** The MicrobiomeGWAS pipeline was applied to 106,912 immune SNPs passed QC and MAF filtering steps. We identified one significant (FDR q-value < 0.1) association (for the chr5:721791) using Jaccard binary dissimilarity and 16 associations (rs4481881, rs1020822, rs11130380, rs16928058, 12 SNPs near the HLA locus) with weighted UniFrac distances. Across the distance methods, the p-value estimations are consistent within groups of weighted/unweighted distance measures.

**Conclusions:** we identified 17 candidate SNPs that influence the microbiome structure. Overall the effect of genetics on the microbiome is moderate.

#### P16.48

#### Host genetics have a moderate influence on the gut microbiome composition

M. Bonder<sup>1</sup>, A. Kurilshikov<sup>1</sup>, P. Deelen<sup>1</sup>, E. F. Tigchelaar<sup>1</sup>, M. Schirmer<sup>2</sup>, T. Vatanen<sup>2</sup>, S. Smeekens<sup>3</sup>, Z. Mujagic<sup>3</sup>, L. Joosten<sup>3</sup>, D. Jonkers<sup>3</sup>, M. Netea<sup>3</sup>, R. Xavier<sup>2</sup>, J. Fu<sup>1</sup>, L. Franke<sup>1</sup>, C. Wijmenga<sup>1</sup>, A. Zhernakova<sup>1</sup>;

<sup>1</sup>University Medical Center Groningen, Groningen, Netherlands, <sup>2</sup>The Broad Institute of MIT and Harvard, Boston, MA, United States, <sup>3</sup>Maastricht University Medical Center, Maastricht, Netherlands, <sup>4</sup>Radboud University Medical Center, Nijmegen, Netherlands.

**Introduction:** A recent twin study has suggested that microbial composition in human's gut is heritable and can be affected by the genetics of the host. However, up to date very few association was detected at single SNP level. In this study we related host genetic variation to the bacterial abundance and function in the human gut in 1,561 individuals.

**Materials and Methods:** Using imputed SNP genotypes and metagenomics sequencing data of three Dutch population cohorts, LifeLines-DEEP (n=1021), 500Fg (n= 435), MIBS (n=105), we linked genome wide SNPs to the abundance of bacteria (bQTLs) and activity of bacterial pathways (bpQTLs). We used the LifeLines-DEEP cohort as a discovery dataset and replicated the associations between SNPs and bacterial taxonomies or pathways in the two other datasets.

**Results:** In the LifeLines-DEEP cohort we identified 9,423 suggestive bQTLs and 5,192 suggestive bpQTLs at  $P < 5 \times 10^{-5}$ . None of the bQTLs were significantly replicated, but we do observe a trend of low p-values. Five of the top bQTL combinations reach genome wide significance in a meta-analysis over all three cohorts.

**Conclusions:** Even though the gut microbiome is shown to be heritable we observed that the overall effect of single SNPs on microbiome is modest and observe a weak replication of the relations between species and pathways

in the gut microbiome and host genetics. We believe a roughly twice as large sample size is necessary to identify the complex relations between the host and microbiome composition and function.

#### P16.49

#### MIPPIT: a fully automated tool to analyse MIPs sequencing data

B. de Koning<sup>1</sup>, M. van de Vorst<sup>2</sup>, S. Castelein<sup>2</sup>, D. Zafeiropoulou<sup>2</sup>, M. Nelen<sup>2</sup>, A. Hoischen<sup>2</sup>, W. van Dijk<sup>1</sup>, L. Claes<sup>1</sup>, A. van den Wijngaard<sup>1</sup>, C. Gilissen<sup>2</sup>;

<sup>1</sup>Maastricht UMC+, Maastricht, Netherlands, <sup>2</sup>Radboudumc, Nijmegen, Netherlands.

Molecular inversion probes (MIPs) is a target enrichment technology that allows simultaneous screening of 1 to 80 genes in large cohorts at a cost of less than €1,- per gene per sample. However analysis of resulting data is complicated by intrinsic properties of MIPs. This limits current freely available software for robust analysis of MIPs data.

Therefore MIPPIT was developed: a fully automated JAVA based tool that enables fast, and robust analysis of MIPs data. It runs multi-threaded on a single node or workstation with a minimum of configuration. During mapping, using the BWA algorithm, reads are subsequently demultiplexed using barcode sequences, stripped of probe sequences in a probe specific manner, and deduplicated per individual sample on the basis of random tags. The resulting BAMs are automatically used for multi-sample variant calling using GATK, and annotated using gencode, dbSNP, ExAC, and several other publicly available data sources. MIPPIT supports variable target lengths, and can handle different strategies for random tag incorporation.

To test MIPPIT, it was used to detect variants within a dataset of 75 samples sequenced for BRCA mutations. Running MIPPIT took 7.5 hours on a 8 CPU computing node. Outcomes were compared to results obtained using commercial software (seqNext). MIPPIT reported all variants detected by seqNext, however in addition it also reported some variants that were overlooked by the latter.

To conclude we believe that MIPPIT is able to analyse MIPs sequencing outcomes in an efficient, reliable and robust manner.

#### P16.50

#### Altered gene expression associated with microRNA binding site polymorphisms

U. Võsa<sup>1</sup>, T. Esko<sup>2</sup>, S. Kasela<sup>2</sup>, T. Annilo<sup>2</sup>;

<sup>1</sup>University Medical Center Groningen, Department of Genetics, Groningen, Netherlands,

<sup>2</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia.

Allele-specific gene expression associated with genetic variation in regulatory regions can play an important role in the development of complex traits. We hypothesized that polymorphisms in microRNA (miRNA) response elements (MRE-SNPs) that either disrupt a miRNA binding site or create a new miRNA binding site can affect the allele-specific expression of target genes. By integrating public expression quantitative trait locus (eQTL) data, miRNA binding site predictions, small RNA sequencing, and Argonaute crosslinking immunoprecipitation (AGO-CLIP) datasets, we identified genetic variants that can affect gene expression by modulating miRNA binding efficiency. For the first time, all identified MRE-SNPs were classified based on whether the allelic direction from eQTL analysis was supported by the logic of miRNA-mediated regulation (concordant or C-type associations) or not (unconcordant or U-type associations). No significant overrepresentation of C-type associations was observed from our analyses (Chi-square test,  $P > 0.05$ ). However, some of the most suggestive eQTL-associated MRE-SNPs locate in the miRNA response elements (MREs) that are supported by experimental evidence.

We also identified MRE-SNPs located in regions associated with complex traits, indicating possible causative mechanisms associated with these loci. The results of this study expand the current understanding of gene expression regulation and help to interpret the mechanisms underlying eQTL effects. This study was supported by the Center of Excellence in Genomics (EXCEGEN) and University of Tartu (SP1GVARENG), EU FP7 grant 306031 (Bestaging), Estonian Research Council Grant IUT20-60. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

#### P16.51

#### miRNA-mRNA prediction tools: use or forget?

O. M. Plotnikova<sup>1</sup>, D. A. Zubtsov<sup>1</sup>, M. Y. Skoblov<sup>1,2</sup>;

<sup>1</sup>Moscow Institute of Physics and Technology, Moscow, Russian Federation, <sup>2</sup>Research Centre for Medical Genetics, Moscow, Russian Federation.

**Introduction:** miRNAs play a key role in regulation of gene expression. Nowadays it's known more than 2'500 miRNAs, but the question about the

identification of miRNA-mRNA interaction is still open. There are five widely used miRNA-mRNA interaction predictive tools: TargetScan, Pictar2, PITA, RNA22 and miRanda. It was impossible to understand if can we trust these predictions. However recently the new method «CLASH» was developed to identify all miRNA-mRNA interactions.

**Materials and Methods:** We created algorithm to compare experimental data from "CLASH" experiment with predicted miRNA sites by all five algorithms from "StarBase" database. Expression data for mRNA and miRNA were used from FANTOM5 and GEO.

**Results:** We estimated working of miRNA-mRNA prediction programs by the following criteria: sensitivity, positive predicted value, predictions in different mRNA regions (3'UTR, CDS, 5'UTR), predictions for different types of interactions (5 classes), predictions of "canonical" and "nocanonical" interactions, and testing by using random data for miRNA binding sites. We compared miRNA binding sites predicted by programs with "CLASH" data and we found 602 miRNAs: 218 were observed only in "CLASH" data, 216 - only in predicted data and 168 - either in "CLASH" and predicted data. Expression analyze of miRNA revealed several interesting groups: highly expressed miRNAs without any interactions, highly expressed miRNAs with small number of interactions and lowly expressed miRNAs, which take a part in a lot of interactions with mRNAs.

**Conclusions:** Comparing miRNA-mRNA prediction programs revealed that all tools generate mostly the same result, which is still far away from experimental data.

#### P16.52

#### Identification of candidate gene pathways for Müllerian duct anomalies by transcriptome profiling of murine mesonephric tissue

*M. Ruiz-Rosario<sup>1,2</sup>, A. Marmolejo-Valencia<sup>3</sup>, E. Martínez-Peñaifiel<sup>1</sup>, R. Ruiz-Esparza Garrido<sup>4</sup>, A. C. Velázquez-Wong<sup>4</sup>, O. Sepúlveda-Robles<sup>3</sup>, S. H. Kofman<sup>5,1</sup>, D. J. Arenas-Aranda<sup>6</sup>, H. Merchant-Larios<sup>7</sup>, F. Fernández<sup>1</sup>;*

<sup>1</sup>Laboratorio de Sistemas, Unidad de Genética, Hospital General de Mexico, Mexico, Mexico, <sup>2</sup>Regional Center For Biomedical Research UCLM, Albacete, Spain, <sup>3</sup>Instituto de Investigaciones Biomédicas, UNAM, Mexico, Mexico, <sup>4</sup>Unidad de Investigación en Genética Humana, CMN Siglo XXI, IMSS, Mexico, Mexico, <sup>5</sup>Facultad de Medicina, UNAM, Mexico, Mexico.

**Introduction:** More than 1 in 4500 women suffer from Müllerian duct anomalies (MDA), which are often associated with renal, skeletal or cardiac malformations. The etiology of these conditions is unknown, and the molecular basis of the female reproductive tract (FRT) differentiation is not fully understood. Murine FRT morphogenesis begins in embryonic day E13, in which the mesonephros harbours both Wolffian and Müllerian ducts, and concludes in E17 with fully differentiated FRT and regressed Wolffian ducts.

**Materials and Methods:** In order to identify genes involved in the morphogenesis of the FRT, we performed microarray analysis of global RNA expression of CD-1 male and female murine mesonephric tissue, during the embryonic days E13 and E15. For each assay, three biological replicates were pooled and microarrays were performed by duplicate.

**Results:** Differential expression patterns were observed among age and gender, and an E15-female signature was identified, corresponding to genes expressed at the midpoint of the FRT differentiation window. Wnt ( $p=7.8e-05$ ) and TGF-Beta ( $p=7.1e-05$ ) signalling pathways, among other biological processes, were statistically overrepresented in these data. For instance, WNT4 mutations were previously associated to a hyperandrogenic form of Müllerian aplasia in humans. Other components of such pathways participate in MDA, formation of limbs, and cardiac abnormalities in mice, suggesting that these mutant phenotypes in humans may result from functionally coherent orthologous gene sets.

**Conclusions:** Our results will be useful to prioritize candidate genes for MDA and will contribute to the current knowledge on FRT morphogenesis. This project was supported by CONACYT-133273 and UNAM-IN219912 grants.

#### P16.53

#### CentoMD®, a comprehensive genotype-phenotype database for rare diseases

*D. Trujillano;*

*Centogene AG, Berlin, Germany.*

Our ability to discover genetic variants in a patient runs far ahead of our ability to interpret them. Databases with accurate descriptions of the causal relationship between the variants and the phenotype are valuable since these are critical tools in clinical genetic diagnostics. Here we introduce CentoMD®, a comprehensive genotype-phenotype database with the main focus on rare diseases. CentoMD® is a browser based software that enables access to a comprehensive, curated, and growing repository of genetic

and clinical information. Its main goals are to aid professionals in the evaluation of genetic variants, to enhance the validity of the genetic analytical workflow, and to facilitate genetic diagnosis and the evaluation of treatment options for patients with hereditary diseases. CentoMD® correlates the clinical information of consented patients and probands of different ethnic backgrounds (>90 countries) with a large dataset of genetic variants and biomarkers (when available). More than 61,000 genetically screened individuals are documented in CentoMD®, resulting in more than 120 million variant detections. Approximately 55% of the mutations in CentoMD are novel. CentoMD® is also a valuable research tool that could allow and facilitate the identification of new disease genes by correlating novel genetic variants with specific, well defined phenotypes.

#### P16.54

#### MUText: a bioinformatic tool to validate human mutations in HGVS standard format

*E. Rizzo<sup>1,2</sup>, I. Limongelli<sup>1,2,3</sup>;*

<sup>1</sup>enGenome srl, Pavia, Italy, <sup>2</sup>Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy, <sup>3</sup>IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy.

Molecular laboratories make use of commercial and/or public resources to build up internal knowledge databases of genomic variants in order to improve classification of pathogenic mutations. Matching mutations identified by a laboratory and those collected by these resources is often challenging, due to different formats used.

Human Genome Variation Society (HGVS) established therefore a standard format to improve knowledge sharing of genomic variants. However, even implementing HGVS recommendations can be error prone, especially if variants are manually curated or specific genomic databases (e.g. transcript subversions) are not used.

For this reasons, we developed MUText, a bioinformatic tool freely available online (<http://engenome.com/mutext>) that checks and converts mutations represented in HGVS v2.0 format.

MUText not only performs a syntax check of variants according to HGVS guidelines, but also tests the consistency of each variant with respect to a specific transcript subversion that can be chosen after selecting a reference genome and a transcript database (e.g. RefSeq and Gencode for GRCh37/h38). MUText returns error messages highlighting these inconsistencies by checking if the replaced nucleotides match the reference genome in the corresponding genomic positions. Variants are then converted to genomic coordinates and formatted according to ANNOVAR in order to be easily integrated in a custom database.

We tested MUText on the Retinoblastoma LOVD ([http://rb1-lsdb.d-lohmann.de/home.php?select\\_db=RB1](http://rb1-lsdb.d-lohmann.de/home.php?select_db=RB1)) reporting 1734 unique variants on RB1 gene (NM\_000321.2) in HGVS format. Errors were reported for 103 mutations: 48 did not match the genomic reference with the replaced nucleotides while 55 were not properly formatted according to HGVS.

#### P16.55

#### Robust method to genotype large ancestral chromosomal inversions using NGS data

*C. Ruiz-Arenas<sup>1</sup>, L. Pérez-Jurado<sup>2</sup>, J. González<sup>1</sup>;*

<sup>1</sup>CREAL, Centre for Research in Environmental Epidemiology, ISGlobal, Barcelona, Spain, <sup>2</sup>Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

**Introduction:** Chromosomal inversions, rearrangements where a fragment of DNA become reversely oriented, can explain part of diseases' genetic causes. Whereas small inversions can be detected using NGS techniques, detection of large chromosomal inversions requires cytogenetic techniques that are not suitable for a high throughput scanning of the samples. Large ancestral chromosomal inversions generate regions with lower recombination rates resulting in the formation of haplotypes. This information can be used to cluster individuals in different inversions genotypes using data from SNP arrays. One of the main limitations of this approach relies on large sample size and SNP coverage requirements. This can be overcome by using NGS data.

**Material and methods:** Differences between the consensus sequence of the standard and the inverted haplotype are used to properly genotype inversions using NGS data. Whole genome sequencing and SNPs array data from 1000 Genomes project have been used to demonstrate our method's performance. When consensus reference is not available, we propose an algorithm that can infer the consensus reference for the different haplotypes using the variants found in the samples.

**Results:** We have applied our method to inversions 17q21.31, 8p23.1 and

16p11 in 503 European samples. Our results have been compared with invClust, a standard tool to detect inversion haplotypes using SNP array data, showing high concordance.

**Conclusions:** Our proposed method is useful to genotype large chromosomal inversions at individual and population level. This can be used in genomic studies which are using NGS as standard genotyping method.

## P16.56

### A likelihood ratio based method to predict exact pedigrees for complex families from next-generation sequencing data

V. Heinrich<sup>1</sup>, T. Kamphans<sup>2</sup>, P. N. Robinson<sup>3</sup>, P. M. Krawitz<sup>2</sup>;

<sup>1</sup>Max Planck Institute for Molecular Genetics, Berlin, Germany, <sup>2</sup>Smart Algos, Berlin, Germany, <sup>3</sup>Institute for Medical Genetics and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany.

Next generation sequencing (NGS) technology considerably changed the way we screen for pathogenic mutations in rare Mendelian disorders.

However, the identification of the disease-causing mutation amongst thousands of variants of partly unknown relevance is still challenging and efficient techniques that reduce the genomic search space play a decisive role. Usually segregation- or linkage analysis are used to prioritize candidates, however, these approaches require correct information about the degree of relationship among the sequenced samples.

For quality assurance an automated control of pedigree structures and sample assignment is therefore highly desirable in order to detect label mix-ups that might otherwise corrupt downstream analysis.

We developed an algorithm based on likelihood ratios that discriminates between different classes of relationship for an arbitrary number of genotyped samples.

By identifying the most likely class we are able to reconstruct entire pedigrees iteratively, even for highly consanguineous families.

We tested our approach on exome data of different sequencing studies and achieved high precision for all pedigree predictions.

By analyzing the precision for varying degrees of relatedness or inbreeding we could show that a prediction is robust down to magnitudes of a few hundred loci.

## P16.57

### Proposals for improvement of next-generation sequencing (NGS) pipeline validations in diagnostic applications

F. Scharf, L. Laner, A. Nissen, A. Benet-Pages, E. Holinski-Feder;

MGZ - Medical Genetics Center, Munich, Germany.

Validation and assessment of variant calling pipelines for NGS in routine diagnostics is a challenging field because of adjustments to specific genetic analyses, different platforms and absence of solid reference datasets. A common approach is to build datasets from Sanger or Microarray data which makes validation time-consuming, expensive and still not reliable. Here we present a detailed evaluation of the validation procedure for routine diagnostic pipelines and suggest useful adaptations.

Concerning reference datasets, the Genome-In-A-Bottle NGS data (NIST-RM8398) is suitable and offers more high-confidence variant calls compared to the 1000Genomes data. Large discrepancies between 1000Genomes and our data required additional Sanger sequencing for clarification (90% of discordant calls were in fact FPs and FNs from 1000Genomes). For statistical evaluation, we propose to use the positive predictive value instead of analytical specificity, which is misleading due to the disproportion of TNs to the number of variants. We performed a detailed analysis of different filter settings for variant calling to determine which parameters have the most influence on FP and FN calls. Additionally, we set thresholds for coverage and quality adapted to each type of variant. It is crucial to determine and report the limitations of the pipeline because of pseudogenes, paralogues and repeat sequences. These low-confidence regions need to be identified for further verification of variant calls with other methods if necessary. Finally, re-validation should be scheduled periodically and not only if relevant changes are implemented, thereby preventing possible failures through unnoticed third-party software changes.

## P16.58

### Meta-alignment: Combining multiple sequence aligners to improve alignment quality

B. Wolf<sup>1,2</sup>, P. Kuonen<sup>1</sup>, T. Dandekar<sup>3</sup>, D. Atlan<sup>4</sup>;

<sup>1</sup>University of applied sciences and arts western switzerland, Villars-sur-Glâne, Switzerland, <sup>2</sup>University of Würzburg, Würzburg, Switzerland, <sup>3</sup>University of Würzburg, Würzburg, Germany, <sup>4</sup>Phenosystems SA, Wallonia, Belgium.

**Introduction:** Many different tools have been developed to perform sequence alignment, a central part of NGS data analysis. Choosing the right tool with the right parameters for a given dataset is a difficult task. Often different aligners perform better than others on parts of the same dataset, making it hard to choose the right tool and configuration.

**Materials and Methods:** We propose a new approach called meta-alignment, which combines the output of multiple sequence aligners to build the best possible alignment. Based on a score matrix, the best possible alignment for every aligned sequence is chosen among the different aligners.

Our approach has been tested on multiple simulated datasets, comparing the results of multiple sequence aligners with the results of meta-alignment which combines their individual results.

**Results:** We could show that combining the output of multiple aligners increases the amount of aligned sequences as well as increasing the amount of correctly aligned sequences.

This result especially holds true if the sequencing data contains a high amount of errors.

**Conclusion:** We could show that at the expense of analysis time, the result of sequence alignment can be improved by combining the results of multiple sequence aligners. The results are consistent throughout the different tests, and are especially pronounced for datasets with high error rates.

## P16.59

### Highlander: variant filtering made easy

R. Helaers, M. Vakkula;

Laboratory of Human Molecular Genetics, De Duve Institute (Université catholique de Louvain), Brussels, Belgium.

A substantial amount of data is being produced by NGS at ever-increasing rates. The technology generates considerable numbers of false positives, and their differentiation from true mutations is difficult. Moreover, the identification of changes-of-interest among thousands of variants requires annotation from various sources and advanced filtering capabilities. We developed *Highlander*, a Java software coupled to a local database, in order to centralize all variant data and annotations, and to provide powerful filtering tools that are easily accessible to the biologist. Data can be generated by any NGS machine and most variant callers. Variant calls are annotated using DBNSFP (providing predictions from 6 different programs, splicing predictions, prioritization scores from CADD and VEST, MAF from 1000G and ESP), ExAC, GoNL and SnpEff, subsequently imported into the database. The database is used to compute global statistics, allowing for the discrimination of variants based on their representation in the database. The GUI easily allows for complex queries to this database, using shortcuts for criteria such as "sample-specific variants", "variants common to specific samples" or "combined-heterozygous genes". Users can browse through query results using sorting, masking and highlighting of information. *Highlander* also gives access to useful additional tools, including visualization of the alignment, an algorithm that checks all available alignments for allele-calls at specific positions, and a module to explore the 'variant burden' gene by gene. *Highlander* is Open-Source (available at <http://sites.uclouvain.be/highlander/>) and also used by genetic centers of two university hospitals in Brussels, as well as in the BridgeIris project (<http://bridgeiris.ibsquare.be/>).

## P16.60

### Improved infrastructure, user interface and genomic mapping in the NHGRI-EBI genome-wide association study (GWAS) catalog

J. Morales<sup>1</sup>, T. Burdett<sup>1</sup>, M. Cerezo<sup>1</sup>, F. Cunningham<sup>1</sup>, L. Gil<sup>1</sup>, P. N. Hall<sup>2</sup>, E. Hastings<sup>1</sup>, L. A. Hindorf<sup>2</sup>, H. A. Junkins<sup>2</sup>, J. A. L. MacArthur<sup>1</sup>, A. Milano<sup>1</sup>, D. Welter<sup>1</sup>, P. Flicek<sup>1</sup>, H. Parkinson<sup>1</sup>;

<sup>1</sup>European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, United Kingdom, <sup>2</sup>Division of Genomic Medicine, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States.

The NHGRI-EBI GWAS Catalog ([www.ebi.ac.uk/gwas](http://www.ebi.ac.uk/gwas)) is a manually curated repository and visual summary of all published GWAS and SNP-trait associations. It was originally created by the NHGRI in 2005 and since 2010, has been produced jointly with EMBL-EBI. The Catalog covers a wide range of human diseases and phenotypes and an expanding number of ancestral backgrounds. As of February 2016, it contains 2,390 studies and 16,775 SNP-trait associations.

To make the GWAS Catalog more intuitive and further increase its utility in identifying and understanding disease loci, the resource has been undergoing a user-focused re-design since 2015. A new infrastructure, curation platform and search interface have been developed to improve functionality, expand scientific content and enhance data display. Specifically, the Catalog now provides enriched ontology-driven search capabilities, accurate display

of complex interaction studies and haplotype analyses, and structured ancestry information for studies published in 2011 and onwards. To complement these efforts, enhancements have been made to the genomic mapping of Catalog data. A new pipeline has been developed to improve mapping quality and provide additional mapping information. This new pipeline is designed to enable future integration of relevant data, such as linkage disequilibrium and regulatory information. The new user interface provides a range of data download options and is fully interactive, with links to related catalog data and to external resources, such as Ensembl.

Ultimately, these developments will improve user-experience and facilitate efforts to identify causal variants and forward our understanding of human disease.

#### P16.62

#### pBRIT: advanced Prioritization of candidate genes using Bayesian Regression & Information-Theoretic approach

A. A. Kumar<sup>1,2</sup>, L. V. Laer<sup>1</sup>, B. Loey<sup>1</sup>, G. Vandeweyer<sup>1,2</sup>;

<sup>1</sup>Department of Medical Genetics, University of Antwerp, Antwerp, Belgium,

<sup>2</sup>Biomedical informatics, Antwerp University Hospital, Antwerp, Belgium.

Identification of top candidate genes from a pool of large data sets is computationally challenging. Existing prioritization tools typically utilize fusion of different annotation sources. However, these tools have important shortcomings. First, outdated annotation sources lead to annotation errors propagating to downstream analysis. Second, data fusion approaches generally fail to address sparsity and dependencies in annotation sources. Finally, high-throughput scalability is limited.

We propose pBRIT, a fast and adaptive tool integrating ten different annotation sources (Pubmed, GO, HPO, Pathway, Interactions, Disease Ontologies, GAD, HuGe, BLAST, Mouse Ontologies) into the prioritization approach. Our hypothesis states that genes involved in similar disease types share similar “functional and phenotype” characteristics that can be used in disease gene identification.

pBRIT is based on an Information-Theoretic approach that models dependencies and sparsity of annotation sources for effective feature mining. Bayesian regression is applied to a training set, consisting of known disease genes, to learn a linear mapping between functional and phenotype annotations. Based on this mapping, candidate genes are ranked according to their relatedness to the training genes.

We validated pBRIT on 1154 genes from 12 different disease classes and compared performance against four existing tools (ChenB et al BMC Med Genomics 2015).

We achieved an average AUC of 0.91 as compared to 0.83 for the best scoring existing tool, indicating excellent sensitivity and specificity. Variation of average AUC score across 12 disease classes is minor, proving robustness of pBRIT. Moreover, pBRIT is fast and can be easily parallelized for analyzing thousands of samples.

#### P16.63

#### A methodology for the reliable analysis of amplicon-based NGS data

S. ZUCCA<sup>1,2</sup>, M. Valente<sup>1</sup>, S. Gagliardi<sup>1</sup>, G. Grieco<sup>1</sup>, J. Garau<sup>1</sup>, M. Plumari<sup>1</sup>, A. Asaro<sup>1</sup>, P. Magni<sup>2</sup>, C. Cereda<sup>1</sup>;

<sup>1</sup>Center of Genomics and post-Genomics, C. Mondino National Institute of Neurology Foundation, Pavia, Italy, <sup>2</sup>Laboratory of Bioinformatics, Mathematical Modelling and Synthetic Biology, Department of Electrical, Computer and Biomedical engineering, University of Pavia, Pavia, Italy.

**Background:** Genetic testing plays an important role in the diagnosis and management of neurological disorders. Next generation sequencing (NGS) has revolutionized the traditional diagnostic approach, by inferring phenotypes directly from genetic testing. Amplicon-based targeted resequencing is a commonly adopted solution for NGS applications focused on specific genomic regions. NGS techniques generate high-throughput genomic data and specific analysis procedures (bioinformatic pipelines) are currently developed to extract the information of interest from the large amount of raw data generated as output.

**Objective:** Definition and validation of bioinformatic pipeline to analyze amplicon-based NGS data managing allele drop-out artifacts.

**Methods:** Seven True Seq Custom Amplicon Illumina gene panels specific for Parkinson Disease, Aicardi-Goutières Syndrome, Intracerebral Hemorrhage Diseases, Familial Hemiplegic Migraine, Leber Congenital Amaurosis and Septo Optic Dysplasia and Ocular Malformation were designed. A total of 309 samples was sequenced (Illumina MiSeq). A customized pipeline has been designed, implemented and validated to analyze data and manage allele drop-out (ADO) artifacts. Results have been compared to state-of-the-art techniques.

**Results:** Panel characterization in terms of coverage, read depth, number of variants found and allele drop-out potential impact has been carried out. Data analysis showed the presence of new non-synonymous mutations and classical pathogenic mutations. The most interesting data concerned the identification of one false negative mutation and 80 false positive, both confirmed via Sanger sequencing.

**Conclusions:** In this work, we focused on ADO-related artifacts and we developed a bioinformatic methodology to manage such issue, in order to maximize the information retrieved from available sequencing data.

#### P16.64

#### Droplet digital PCR (ddPCR)-based validation and genotyping of human inversions

M. Puig<sup>1</sup>, S. Pacheco<sup>1</sup>, D. Izquierdo<sup>1</sup>, J. F. Regan<sup>2</sup>, G. Karlin-Neumann<sup>2</sup>, M. Cáceres<sup>1,3</sup>;

<sup>1</sup>Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, <sup>2</sup>Digital Biology Center, Bio-Rad Laboratories, Pleasanton, CA, United States, <sup>3</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

The last years have seen a great interest in genomic structural variation and despite the difficulty of their study, an increasing amount of information is starting to accumulate about human inversions. However, one important limitation in the case of inversions is the presence of large inverted repeats (IRs) at the breakpoints, which precludes determining the orientation of the intervening region by most simple methods. Here we describe a new linkage-based application of droplet digital PCR (ddPCR) for the validation and genotyping of inversions mediated by IRs. First, we established that the strategy works for a few known human inversions with defined genotypes, including the well-characterized Chr. 17 inversion. Next, we developed ddPCR assays for a total of 18 inversions, with sizes ranging from 6.8 to 747 kb and IRs from 6.3 to 134 kb, and we genotyped them across 15 individuals of diverse origins. Our analysis allowed us to confirm all the tested inversions, except two, for which the inverted orientation was not found in our small sample or the assay could not distinguish both orientations. In addition, by comparing with previous data and independent replicates, we have shown that the technique is highly accurate and reproducible. This work illustrates the power of partitioning in ddPCR for diverse applications. Furthermore, it makes it possible for the first time to screen quickly a large number of samples to assess the potential functional effects and clinical implications of these rearrangements.

**Support:** European Research Council (ERC) Starting Grant (INVFEST) and Bio-Rad Laboratories.

#### P16.65

#### A novel unsupervised clustering approach with multiple data types to reveal fine-level structure

K. Chaichoompu<sup>1,2</sup>, S. Tongsim<sup>3</sup>, P. J. Shaw<sup>4</sup>, A. Sakuntabhai<sup>5,6</sup>, K. Van Steen<sup>1,2</sup>;

<sup>1</sup>GIGA-R Medical Genomics, University of Liege, Liege, Belgium, <sup>2</sup>Montefiore Institute, University of Liege, Liege, Belgium, <sup>3</sup>Genome Technology Research Unit, National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand, <sup>4</sup>Medical Molecular Biology Research Unit, National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand, <sup>5</sup>Functional Genetics of Infectious Diseases Unit, Institut Pasteur, Paris, France, <sup>6</sup>Centre National de la Recherche Scientifique, Paris, France.

**Introduction:** Several methods exist to identify population substructure that is due to shared genetic ancestry or regional proximity. These may be SNP-based or haplotype-based (Price et al. 2006, Lawson et al. 2012). Here, we present a flexible unsupervised clustering approach that is built on the ipPCA machinery (Intarapanich et al. 2009).

**Methods:** Our method supports both numeric and categorical data, and can be applied to panels of SNPs and/or haplotypes, or gene-based integrative summaries (Fouladi et al. 2015). Unlike ipPCA, our method involves an iterative process using binary and ternary splits based on multivariate Gaussian mixture modeling of PCs and Clustering EM (CEM) estimation as in (Lebret et al. 2015). To assess performance, we considered different simulated scenarios of  $F_{ST}=[0.0005, 0.006]$ , 5,000-20,000 independent SNPs in HWE, 500-8,000 individuals, and 2-4 populations (Balding and Nichols 1995), with 100 replicates for each scenario. SNPs were treated as categorical or continuous (including ancestry-corrected SNPs). Haplotype-based runs used HapMap 3 data: CHB-JPT ( $F_{ST}=0.007$ ) and CEU-TSI ( $F_{ST}=0.004$ ).

**Result and Conclusion:** In simulated scenarios of extremely subtle structure ( $F_{ST}=[0.0009, 0.002]$ ), a population classification accuracy of 92.56% or greater was obtained, which was superior to ipPCA. Promising results to detect fine structure were also obtained in case of the HapMap populations. We believe that the ability of our approach to detect subtle structure, including outlier individuals, will be important in molecular reclassification studies of patients from whom underlying population patterns have been

removed.

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## P16.66

### Genotyping of all public RNA-sequencing data for large scale trans-QTL and ASE studies

*N. P. de Klein<sup>1</sup>, F. van Dijk<sup>1,2</sup>, P. Deelen<sup>1,2</sup>, M. Bonder<sup>1</sup>, J. Karjalainen<sup>1</sup>, P. Folkertsma<sup>1</sup>, F. Kelpin<sup>1,2</sup>, J. Jetten<sup>1,2</sup>, A. Claringbould<sup>1</sup>, M. Swertz<sup>1,2</sup>, L. Franke<sup>1</sup>,*

<sup>1</sup>University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands, <sup>2</sup>University of Groningen, University Medical Center Groningen, Genomics Coordination Center, Groningen, Netherlands.

Genome wide association studies (GWAS) and expression quantitative trait locus (eQTL) analyses have recently identified hundreds of disease- and expression-associated genetic loci. However, finding the causal SNP in a locus remains challenging, especially for rare variants. Additionally, studies show that eQTL effects are often cell type and tissue specific, so there is a need for large numbers of cell type specific samples. We have previously shown that public RNAseq data from 9500 samples can be used to identify eQTL effects for 8,034 unique genes (Deelen et al., 2015). By analyzing public RNAseq samples at an even larger scale, we will further increase our detection power.

For this purpose we developed a pipeline to genotype and quantify gene expression for all RNAseq samples in the European Nucleotide Archive (ENA). In August we retrieved 33,000 samples from ENA, which includes, among others, eight tissues with each over 500 samples. Of the 33,000 quantified samples, 9,000 have been haplotype-called and approximately 5,000 are of sufficient quality to continue with genotyping.

This strictly quality controlled re-use of freely available data will allow us to find many previously undetected allelic imbalanced SNPs and eQTLs. Moreover, we expect the sample size to be large enough to also find tissue specific trans-eQTLs and to contribute meaningfully in large scale trans-eQTL or ASE studies. By overlapping eQTLs with other sources of SNP information, such as methylation QTLs and DNAseq QTLs, we can assist in the interpretation and prioritisation of likely pathogenic variants.

## P16.67

### GenCor - a tool for quality control and validation of whole-exome sequencing data through incorporation of array-based genotyping

*S. Sivalingam<sup>1</sup>, A. Hofmann<sup>1</sup>, S. Herms<sup>2,1</sup>, T. Kätsel<sup>1</sup>, K. U. Ludwig<sup>1</sup>, S. Heilmann-Heimbach<sup>1</sup>, A. J. Forstner<sup>1</sup>, M. M. Nöthen<sup>1</sup>, P. Hoffmann<sup>2,1,3</sup>;*

<sup>1</sup>Institute of Human Genetics; Department of Genomics; Life & Brain Center; University of Bonn, Bonn, Germany, <sup>2</sup>Human Genomic Research Group, Department of Biomedicine, University Hospital and University of Basel, Basel, Switzerland, <sup>3</sup>Institute of Neuroscience and Medicine; Research Center Jülich, Jülich, Germany.

Whole-exome sequencing (WES) is the targeted sequencing of the protein-coding regions of the human genome. It is routinely used in human genetic studies and is a cost-efficient method for identification of rare variants. Many bioinformatics pipelines have been developed for variant calling in WES data. The performance of these pipelines depends on the applied algorithms and the implemented filter strategies. Given the complexity of large scale sequencing projects sample mix-ups can interfere with the final results. Here we present GenCor, a tool for quality control of WES data by incorporation of array-based genotyping.

For assessing the quality of WES data, GenCor calculates the genotype concordance between WES called variants and array-based genotypes. For the evaluation of GenCor, WES data from five human DNA samples were used. Exome capture was performed using SureSelectXT Human AllExon V5. Libraries were sequenced on a HiSeq2500 with 2x125 bp. Raw data were processed using the default BWA/GATK v3.4 pipeline. The same set of samples was processed on Infinium CoreExome-24 BeadChip that comprises >269,000 markers.

In our data set no sample mix-ups in WES preprocessing could be identified. For variant quality control, an average agreement of 10,135 variants per sample between WES variants and array-based genotypes was observed. In total, 99% concordance of WES variants and array-based genotypes were found. We recommend the incorporation of array-based genotyping for improved quality control in WES projects. The GenCor tool can also be applied to whole-genome sequencing and is in principle compatible with genotype data from other genotyping platforms.

## P16.68

### Framework for quality assessment of whole genome, cancer sequences

*J. P. Whalley<sup>1,2</sup>, K. Kleinheinz<sup>3,4</sup>, E. Rheinbay<sup>5,6</sup>, K. Raine<sup>7</sup>, S. Waszak<sup>8</sup>, J. Gehring<sup>9</sup>, D. Huebschmann<sup>3,4,9</sup>, M. Schlesner<sup>3,4</sup>, M. D. Stobbe<sup>1,2</sup>, J. Werner<sup>3,4</sup>, J. Jennings<sup>10</sup>, J. Korbel<sup>10</sup>, P. Campbell<sup>11</sup>, G. Getz<sup>5,6,11</sup>, I. Buchhalter<sup>3,4</sup>, I. G. Gut<sup>1,2</sup>, Pan-Cancer Analysis of Whole Genomes (PCAWG);*

<sup>1</sup>CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, Barcelona, Spain, <sup>3</sup>Division of Theoretical Bioinformatics (B080), German Cancer Research Center (DKFZ), Heidelberg, Germany, <sup>4</sup>Department for Bioinformatics and Functional Genomics, Institute for Pharmacy and Molecular Biotechnology (IPMB) and BioQuant, Heidelberg University, Heidelberg, Germany, <sup>5</sup>Massachusetts General Hospital Cancer Center and Department of Pathology, Boston, MA, United States, <sup>6</sup>Broad Institute of Harvard and MIT, Cambridge, MA, United States, <sup>7</sup>Wellcome Trust Sanger Institute, Hinxton, United Kingdom, <sup>8</sup>European Molecular Biology Laboratory, Heidelberg, Germany, <sup>9</sup>Department of Pediatric Immunology, Hematology and Oncology, University Hospital Heidelberg, Heidelberg, Germany, <sup>10</sup>Ontario Institute for Cancer Research, Toronto, ON, Canada, <sup>11</sup>Harvard Medical School, Boston, MA, United States.

The Pan-Cancer Analysis of Whole Genomes (PCAWG) project has put together a cohort of nearly 3000 cancer whole genomes from 47 projects covering 20 different tissues. All of this data is processed in a homogeneous way, primarily to identify somatic mutations, allowing a wide range of analyses within and across cancer types. However, as the sequencing was done by different sequencing facilities, with varying protocols and over a long period of time, careful attention must be paid to the quality of the genomes.

To capture the quality of the genome sequences provided, the PCAWG Quality Control working group developed a framework to assess the quality and provide a succinct summary for PCAWG. The framework consists of five different measures of quality and a star rating system. The measures computed, for both the normal and the tumour genome, are the mean coverage, the evenness of coverage, somatic mutation calling power, paired reads mapping to different chromosomes, and paired reads of which one read fails to map. We summarised these into a star rating, with five stars representing the highest quality cancer genomes, passing all thresholds set for each measurement, and the rest on a descending scale in half star steps. The star rating system provides an easy to capture description of the quality of the sequences and flags problematic samples that should be eliminated from downstream use. We believe that this is an effective framework of quality measures for similar large-scale studies that look to use sequences from different sources.

## P16.69

### Interactive software for the integrated analysis and identification of rare and undiagnosed diseases using NGS data

*S. Pabinger<sup>1</sup>, D. Katic<sup>2</sup>, A. Krolo<sup>3</sup>, T. Hirschmugl<sup>1</sup>, K. Boztaug<sup>3,4,5</sup>, A. Kriegner<sup>2</sup>, K. Vierlinger<sup>1</sup>;*

<sup>1</sup>AIT Austrian Institute of Technology, Vienna, Austria, <sup>2</sup>Platomics, Vienna, Austria,

<sup>3</sup>CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, <sup>4</sup>Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria, <sup>5</sup>CeRUD Vienna Center for Rare and Undiagnosed Diseases, Vienna, Austria.

The growing number of known rare diseases is estimated to be larger than 7,000, with more being discovered each day. Recent advances in next-generation sequencing (NGS) have revolutionized genomics allowing physicians and scientists to examine patients at an unprecedented. Nowadays, the challenge with NGS technologies is the meaningful interpretation and analysis of data.

Here we present an integrated solution that facilitates analysis and identification of potential causing mutations in rare and undiagnosed diseases from NGS-based genome sequencing studies. All complex analysis steps are abstracted from the end user and results are presented in an intuitive and interactive way. The application annotates identified variants with over 50 properties, including descriptive statistics, prediction scores, frequencies from public databases, and information from disease related databases. An interactive filtering, prioritization, and classification mechanism is included offering new ways to analyze variants. Family studies can be collectively analyzed using sophisticated querying and filtering methods including graphical representations of the relationships. The whole system is available as a web-based application integrated into the Platomics platform, which offers powerful features for heterogeneous data handling of samples, and performing analysis runs using optimized parameter settings.

As a proof of principle, using the new software we have analyzed targeted sequencing data in a cohort of patients with primary immunodeficiency disorders (PIDs) of unknown molecular origin. The new application will contribute to fast and reliable diagnosis, which could have a major impact for the PID patient treatment, as well as for personalized medicine.

## P16.70

## Promoting data sharing and aiding variant interpretation in rare genetic disorders using the DECIPHER platform

G. J. Swaminathan, S. Brent, B. Hutton, D. Perrett, P. Bevan, M. Hurles, H. Firth; Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

DECIPHER (<https://decipher.sanger.ac.uk/>) is a collaborative data sharing and interpretation platform that enables the secure upload, analysis and subsequence sharing of anonymised phenotype-linked patient variant data. DECIPHER is a worldwide user community of over 250 clinical genetics centres and research groups from over 40 countries that utilise the built-in tools for aiding the interpretation of variants as well as to discover other patients that share similar phenotype and genomic findings. As a collaborative tool, DECIPHER encourages contact between depositors and mediates contact requests from external users on cases that have been made open-access following informed patient consent. These have resulted in over 1000 peer-reviewed publications in scientific literature using DECIPHER data since 2010, leading to new discoveries that further understanding of gene-phenotype relationships. DECIPHER is also a founding member of the MatchMaker Exchange (MME) project (<http://www.matchmakerexchange.org/>), an initiative aimed at facilitating the matching of cases across geographically diverse locations and databases using standard procedures and a common application programming interface (API).

As part of our continuing efforts to make phenotype-linked variant data more meaningful in modern clinical genetics, we have developed new visualisation methods and interpretive tools within the DECIPHER platform. These include an interactive phenotype browser to explore and identify gene-phenotype correlations, dosage sensitivity scores for deposited copy-number variants, enabling the capture of extended patient information (growth curves, phenotype modifiers, development milestones) among others. Our presentation will highlight these new developments as well as focus on the implementation of the MME API within DECIPHER for data discovery and collaboration.

## P16.71

## Simple and effective variant filters to complement GATK-VQSR in WES data analysis

A. Larionov<sup>1</sup>, M. Goldgraben<sup>1</sup>, E. Fewings<sup>1</sup>, J. Redman<sup>1</sup>, P. Concannon<sup>2</sup>, J. Bernstein<sup>3</sup>, WE CARE, J. Hadjidakis<sup>4</sup>, D. Conti<sup>5</sup>, M. Tischkowitz<sup>2</sup>;

<sup>1</sup>Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom,

<sup>2</sup>Genetics Institute and Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, United States, <sup>3</sup>Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, United States,

<sup>4</sup>Genomics Core, Cancer Research UK Cambridge Institute, Cambridge, United Kingdom,

<sup>5</sup>Division of Biostatistics, Department of Preventive Medicine, University of Southern California, Los Angeles, CA, United States.

Introduction: Standard settings recommended by GATK-Best-Practices for WES pipelines produce a large number of low quality variants that need further filtering before down-stream analyses. We describe a simple set of filters, which significantly improve variant quality metrics when added to the standard GATK-VQSR procedure.

Methods: Filtering options were compared on 3 WES germline datasets (Nextera-Rapid-Capture, HiSeq2500, PE75-125). Initially, a standard VQSR filtering was performed according to the current GATK-Best-Practices. Then this was complemented by reducing the padding allowance (to 10bp) and by adding two more hard filters for average DP (min 10x) and for QUAL (corresponding to TiTv>2).

Results: are summarised in table

	Dataset-A	Dataset-B	Dataset-C
N of samples	512	42	45
Raw variants called by GATK HC (default settings)	794,680	268,147	300,757
TiTv (SNPs)	2.11	2.35	2.26
Standard VQSR filtering and padding (SNP TS 97%, INDEL TS 95%, padding 100bp)			
N of variants	552,535	227,766	247,421
TiTv (SNPs)	2.31	2.47	2.38
Standard VQSR filtering + set of custom filters			
N of variants	239,100	90,332	108,698
TiTv (SNPs)	2.91	2.89	2.84

Plotting VQSLOD histograms was an informative addition to standard GATK practices. Clear multi-modal VQSLOD distributions confirmed suitability of VQSR for SNP-filtering. In contrast, VQSLOD distributions in INDELS showed that they cannot be successfully filtered by VQSR. Analysis of DP distributions made it clear that GATK-recommended padding (100bp around targets) was arbitrary and excessive. Reducing it to 10bp removed a high number of poor quality variants, while preserving essential splice sites. Distributions and utility of other filters have been also explored.

Conclusion: Three simple and intuitive hard filters added to the standard

GATK-VQSR procedure were sufficient to dramatically reduce the total number of variants and to improve their quality, making the metrics consistent with those expected in WES studies.

Grant information: ERC

## P16.72

## Whole Genome Sequencing of successfully aging long-lived Polish Caucasians and creation of a reference database

A. M. Boguszewska-Chachulska<sup>1</sup>, E. Matczynska<sup>1</sup>, P. Lyszkiewicz<sup>2</sup>, M. Krawczyk<sup>1</sup>, D. Kowalczyk<sup>2</sup>, R. Szymanczak<sup>1</sup>, A. Piotrowska<sup>3</sup>, M. Mossakowska<sup>4</sup>, M. Puzianowska-Kuznicka<sup>5</sup>, M. Zagulski<sup>1</sup>;

<sup>1</sup>Genomed SA, Warsaw, Poland, <sup>2</sup>24 Godziny Sp. z o.o., Warsaw, Poland, <sup>3</sup>Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland,

<sup>4</sup>International Institute of Molecular and Cell Biology, Warsaw, Poland, <sup>5</sup>Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

The aim of the project was to characterise genome sequence variation in the Polish population and to combine it with collected clinical data for diagnostic and scientific applications.

Whole genomes of 130 successfully aging Polish 90+ years old subjects were sequenced with a mean 30x coverage using the Illumina technology. An in-house developed bioinformatic pipeline, including BWA, GATK and VEP, was applied to identify and annotate sequence variants.

The genomic data were combined with a large set of clinical and biochemical records. Mitochondrial genomes were analysed and identified variants, in 780 positions when compared to rCRS, were added to the database. An analysis of structural and copy number variations was performed.

Approximately 21.3 mln variants in the nuclear genome were found, including 16.9 million SNVs and 4.4 million indels. 72% of variants have been already present in the dbSNP database, 58% in the 1000 Genomes Project. Almost 4.0 mln novel SNVs and 2.5 mln novel indels were detected. The search for rare variants categorised by the Human Gene Mutation Database as disease-causing (DM) yielded 764 variants with MAF<0.5%, which corresponds to 6 distinct rare DM variants per each long-lived person.

The results of this project shed a new light on the effect of previously characterised disease-causing mutations on the individual health and life span. The reference database of successfully aging long-lived Caucasians constitutes a valuable tool for the clinical and prophylactic applications in the field of personalised medicine.

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## P16.73

## Somatic retroelement insertions identification by the new normalization-based method

A. A. Minervina<sup>1</sup>, A. Y. Komkov<sup>1</sup>, A. A. Kurnosov<sup>1</sup>, V. I. Nazarov<sup>1,2</sup>, M. V. Pogorelyy<sup>1</sup>, K. V. Khodosevich<sup>3</sup>, I. Z. Mamedov<sup>4</sup>, Y. B. Lededev<sup>1</sup>;

<sup>1</sup>Shemyakin-Ovchinnikov Institute of bioorganic chemistry, Moscow, Russian Federation,

<sup>2</sup>National Research University Higher School of Economics, Moscow, Russian Federation,

<sup>3</sup>Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark.

Introduction: Recent studies have shown that retrotransposition activity is not limited to germline cells, but is also typical for somatic cells. Most research on somatic retrotransposition are focused on brain, since retroelement (RE) insertions are speculated to contribute to neurons plasticity. Whereas somatic RE insertions are very rare, their identification is complicated even with the use of the most robust high-throughput sequencing platforms. Thus, several hybridization and amplification-based methods are used to target sequencing to selected subgroups of REs. However, none of these approaches could provide specific enrichment for somatic insertions. We developed a new normalization-based method to enrich for somatic RE insertions.

Materials and methods: Sequencing libraries were prepared from the DNA sample of 50000 nuclei from human brain cortex. First, the selective amplification with AluYa5-specific primer was performed. The amplicon was then subjected to two rounds of normalization using duplex-specific nuclease. Sequencing data was analyzed by unique bioinformatics pipeline to generate the list of high-confident somatic Alu insertions.

Results: The use of genomic normalization increased the number of fragments, flanking somatic Alu insertions in the sequenced sample by 20-fold, and the number of identified somatic insertions 6,5-fold. We identified 399 somatic insertions, 65 of which were present in more than one cell.

Conclusions: The developed approach could significantly increase the efficiency of somatic REs identification in normal and malignant cells. Moreover, the use of molecular identifiers provides the direct estimation of the number of cells bearing a particular somatic insertion.

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## P16.74

### Identification of Rheumatoid Arthritis specific pathways from exome data in multiplex families.

**M. Veysiere<sup>1</sup>, J. Perea<sup>1</sup>, L. Michou<sup>2</sup>, A. Boland-Auge<sup>3</sup>, V. Meyer<sup>3</sup>, J. Deleuze<sup>3</sup>, F. Cornelis<sup>4</sup>, E. Petit-Teixeira<sup>1</sup>, V. Chaudru<sup>1</sup>;**

<sup>1</sup>GenHotel-EA3886 - Evry University, Evry, France, <sup>2</sup>Division of Rheumatology, CHU de Québec, Department of Medicine, Québec, QC, Canada, <sup>3</sup>Centre National de Génotypage, Genomic Institute, CEA, Evry, France, <sup>4</sup>GenHotel-Auvergne, EA4679, Auvergne University, Genetic Department, CHU Clermont-Ferrand, Clermont-Ferrand, France.

**Introduction:** The genetic component of Rheumatoid Arthritis (RA) is not fully defined. Identification of rare variants through NGS analysis could help us to characterize a part of the missing heritability.

**Materials and Methods:** To identify rare RA-associated variants, we have analyzed exome sequences from 30 individuals (22 RA and 8 unaffected subjects) belonging to families with at least four cases of RA and/or other autoimmune diseases. Exons were captured with Agilent SureSelect Human All Exon V5 kit and sequenced with an Illumina HiSeq2000 sequencer. After alignment, targeting of captured regions, variant calling and quality filtering, we selected variants with allele frequency lower than 1% or absent in databases. We then performed association studies (Fisher test) between RA and pathways enriched in genes containing RA-specific variants (identified with DAVID).

**Results:** We identified 262030 rare variants carried by at least one individual. In order to conduct pathway analysis, we selected variants present in all RA cases of a same family but absent in all other individuals (n=607 variants). The number of variants found in a particular family varied from 12 (2%) to 132 (22%). All the variants, spread across 580 genes, allowed us to identify 2 pathways significantly associated with RA: the cadherin signaling pathway (PFisher=4\*10-3) and the focal adhesion pathway (pFisher=4\*10-2).

**Conclusions:** Analysis will be completed by taking into account the RA status with or without other autoimmune diseases. Selected variants will be further validated by genotyping all available members of each family in order to perform segregation analysis.

## P16.76

### Regulation of transcribed intergenic regions in human substantia nigra and putamen

**M. S. Guelfi<sup>1,2</sup>, J. Vandrocova<sup>1,2</sup>, J. A. Botía<sup>1,2</sup>, K. D'Sa<sup>1,2</sup>, M. E. Weale<sup>2</sup>, J. A. Hardy<sup>1</sup>, M. Ryten<sup>1,2</sup>;**

<sup>1</sup>Department of Molecular Neuroscience, UCL Institute of Neurology, London, United Kingdom, <sup>2</sup>Department of Medical & Molecular Genetics, King's College London, London, United Kingdom.

Expression Quantitative Trait Loci (eQTL) studies are a successful approach to study how genetic variation influences transcription. A current limitation in most eQTL studies is that they rely on annotated references to measure transcript abundance. While existing annotation is likely to be accurate for many human tissues, the complexity of gene expression and splicing in human brain implies that existing references are less accurate, and this view is supported by recent RNA-sequencing (RNA-Seq) analyses. In this study, we investigate regulation of transcribed intergenic regions in two brain tissues: putamen and substantia nigra. We performed RNA-seq on healthy post-mortem brain tissues from the UKBEC dataset to identify and quantify transcribed regions in a reference-agnostic manner with Derfinder R package. After filtering steps, we combined the abundance of the intergenic transcribed regions with genotype information to perform cis-eQTL mapping analysis, detecting 1954 independent cis-acting eQTL signals in putamen and 744 in substantia nigra. Finally, applying exon-junction identification and co-expression analysis, we investigated the independence of these regions from the nearest annotated genes. Using these approaches, we identified the existence of intergenic transcribed regions with high correlation with the nearest gene and shared eQTLs suggesting incomplete annotation of known genes. However, we also identified transcribed intergenic regions with independent eQTLs suggesting the existence of novel regulated transcribed regions. Both types of genomic regions represent as yet unexplored candidate regions for the identification of potentially pathogenic variation. Therefore, these findings are of direct relevance to the prioritisation of variants in clinical diagnostics.

## P16.77

### A critical comparison between Sanger and Next Generation Sequencing

**H. Fridman<sup>1,2</sup>, M. Einhorn<sup>3</sup>, Y. Porat<sup>3</sup>, C. Bormans<sup>4</sup>, A. Bormans<sup>4</sup>, L. Sanchez<sup>4</sup>, E. Levy-Lahad<sup>1,2</sup>, D. Behar<sup>1,3,4</sup>;**

<sup>1</sup>Shaare Zedek Medical Center, Jerusalem, Israel, <sup>2</sup>Hebrew University-Hadassah School of Medicine, Jerusalem, Israel, <sup>3</sup>Data Analysis Group, Genoox, Tel Aviv, Israel, <sup>4</sup>Genomic Research Center, Gene by Gene, Houston, TX, United States.

**Background:** Clinical uses of Next Generation Sequencing (NGS) are continuously expanding. However, unbiased systematic comparisons of performance of NGS and Sanger-sequencing is still lacking. We present a comparative evaluation based on ~1.3% of the exome of a single individual.

**Materials and Methods:** Genomic DNA was extracted from peripheral leukocytes. Sanger-sequencing was performed for 258 genes. Whole exome sequencing (WES) was done on the Illumina HiSeq2500 using the Nextera (FC-140-1006) capture kit (coverage 70x). WES data was analyzed using standard NGS analytic pipelines with predefined quality thresholds.

**Results:** Sanger-sequencing encompassed a total of 4,755 exons spanning 1,212,319 bp. The Nextera capture kit overlaps 99.4% of the coding sequence and 94% of the coding sequence including intron/exon boundaries ( $\pm 8$ bp). Within this extended overlap region, 427 and 430 variants were detected by Sanger-sequencing and NGS, respectively. 419 variants were concordant in both platforms. Of the eight variants identified by Sanger-sequencing only, four were in genomic positions with  $\leq 2$  NGS coverage, and four were not detected despite good coverage. A total of 11 variants were identified only by NGS. Further results from buccal-driven WES and the Agilent (SureSelectQXT) Capture kit will be presented.

**Conclusions:** The overall concordance between Sanger-sequencing and NGS was 98.1% (419/427). On an exome-wide scale, we extrapolate that ~150-200 variants would be discordant. Sanger-only variants may be explained by NGS-coverage failure, either specific or random. NGS-only variants may reflect Sanger-sequencing primer binding-sites polymorphisms, or mosaicism. Single-platform variants may also represent errors. Geneticists should be aware of these limitations.

## P16.78

### Bioinformatics analysis of schizophrenia risk variants: a novel methodology for uncovering potentially causal variants within a locus

**E. Jamieson<sup>1,2</sup>, B. D. Collier<sup>3</sup>, C. D. Marren<sup>4</sup>, D. J. Scherschel<sup>4</sup>, E. Eastwood<sup>4</sup>;**

<sup>1</sup>Eli Lilly and Company, Surrey, United Kingdom, <sup>2</sup>University of Glasgow, Glasgow, United Kingdom, <sup>3</sup>Lilly, Indiana, IN, United States, <sup>4</sup>Lilly, Surrey, United Kingdom.

**Introduction:** Genome-wide association studies can be difficult to interpret because the unit of association is genetic polymorphism which marks regions of the genome rather than specific genes. We present a methodology which combines multiple bioinformatic variant effect prediction tools to create a single score used to identify potentially causal variants within a GWAS locus, to assist the SNP-to-gene mapping process. Materials and methods: A KNIME workflow was used to implement a novel variant-scoring methodology which combines the weighted effects of variants in a locus to produce a list of genes ranked in order of their predicted contribution to schizophrenia risk. Factors used to determine the weighted effect include a GWAS p-value and a PHRED-scaled CADD score. Results: TCF4 and FES, both strongly probably schizophrenia risk genes, appear within the top three positions in the ranked gene list, providing confidence that the method is useful in SNP-to-gene mapping and ranking associations. TCF4 has a large number of non-coding variants in its vicinity, of which only rs143743309 scores highly, whereas FES has three highly-scoring variants in its locus. Two of these are missense variants and one is a frameshift. In this case the frameshift variant is likely to be causal of the association at the locus. Conclusions: This methodology will improve our understanding of what causes a locus to be associated with risk for complex genetic diseases, along with aiding in assessment of which gene is affected to allow us to approach drug target discovery with better validated genetic evidence.

## P16.79

### Optimization of RNA isolation from small volumes of serum to enable small RNA sequencing

**R. Pluvinet<sup>1</sup>, G. Rech<sup>1</sup>, L. Sumoy<sup>1</sup>, IMPPC, Badalona, Spain.**

**Introduction:** Circulating miRNAs have been extensively explored for their use as biomarkers of disease. The aim of the present study was to optimize procedures for RNA extraction from low volume human serum samples for subsequent library preparation for small RNA-seq.

**Materials and Methods:** We compared two different methods of RNA extrac-

tion (Qiagen miRNeasy serum/plasma kit vs Exiqon miRCURY RNA Isolation kit-Biofluids), with different starting serum volumes (600, 400, or 200  $\mu$ L) and different carriers (MS2 RNA vs glycogen). Quality control for hemolysis and intersample normalization was performed with the Exiqon serum QC qPCR panel.

Three different commercially available small RNA-seq library kits were tested (Illumina Truseq small RNA vs New England Biolabs NEBNext Multiplex small RNA Library prep kit vs BiooScientific NEXTflex small RNA sequencing kit). Pooled libraries were size-selected using the Pippin Prep system, quantified by KAPA qPCR and sequenced on an Illumina HiSeq2500 sequencer with 1x50 nt single reads and analyzed with sRNAbench v07\14 software. Results: The best miRNA extraction yield was obtained when 200  $\mu$ L of serum were processed using Exiqon's miRCURY kit. NEBNext library preparation kit produced the highest miRNA representation in small RNA-seq results. Illumina's TruSeq showed the highest rate of mappable reads with a better adjustment in size distribution around the 22nt expected in mature miRNAs.

Conclusions: Adaptation of standard commercial protocols allows quantitative assessment of the circulating miRNAs by small RNA-seq from low volumes of serum. This has application to studies where limited amounts of sample are available.

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#### P16.80

#### High-Throughput Isolation and Selection of Single Cells using the ICELL8™ Single-Cell System

M. Srinivasan, A. Mir;

WaferGen BioSystems, Fremont, CA, United States.

Single-cell RNA-seq analysis has been used extensively to identify new cell types in complex tissues based on gene expression differences. ICELL8™ Single-Cell System utilizes imaging to identify single-cells and selectively process only those single-cells for RNA-seq applications with >98% accuracy. The ICELL8™ System is comprised of a scalable micro-fabricated metal scaffold containing 5,184 nano-wells (ICELL8™ chip), a multi-sample nanodispenser (MSND) that precisely dispenses reagents and fluorescently-labeled cells into the nano-wells, an imaging station to image all 5,184 wells in 3 min per channel, and CellSelect™ software to unambiguously identify and select wells that contain single cells for further processing for RNA-seq applications. The ICELL8™ chip platform is proven for its flexibility in processing up to 8 samples in a single chip as well as batch-processing precious samples (such as freshly-excised human tumors) in up to 10 chips a day to yield ~15,000 cells/day. Using human-mouse mixed species experiments, we will present data to

1. Validate the reproducible dispensing of individual cells at Poisson distribution.
2. Demonstrate CellSelect™ software ability to unambiguously identify single-cell containing wells.
3. Establish the increased diversity of genes captured per cell.

#### P16.82

#### FindTranslocations - a structural variant calling toolkit

J. Eisfeldt<sup>1</sup>, F. Vezzi<sup>2</sup>, D. Nilsson<sup>1</sup>, A. Lindstrand<sup>1</sup>,

<sup>1</sup>Karolinska institutet, Stockholm, Sweden, <sup>2</sup>Stockholms universitet, Stockholm, Sweden.

A structural variant is traditionally defined as a balanced or unbalanced genetic rearrangement larger than 1kb but recent advances in genetic technology has enabled the detection of increasingly smaller rearrangements. In genetic diagnostic, currently applied techniques such as FISH and microarray studies have limited resolution. Massively parallel whole genome sequencing (WGS) is a promising technique that may be used to identify a large proportion of genomic structural variation in a single experiment. However, the detection of structural variants from WGS data is complicated by the vast amount of normal variants and reference errors and currently relies on using multiple variant callers, increasing the overall computational cost. FindTranslocations is a structural variant detection algorithm using discordant read pairs and coverage information to identify balanced and unbalanced structural variants while consuming less than 5 cpu hours per 30X whole genome sample. It contains a built in database function allowing the user to create local variant frequency databases, that may be used to filter out rare variants or detect variants that are common within a group. FindTranslocations goes beyond mere structural variant calling providing positional information for specific rearrangements to help make a correct interpretation of the clinical significance. We have used FindTranslocations in a validation set of 61 samples with previously identified clinically relevant structural variants for which we have 30x WGS data. FindTranslocati-

ons has a 89% sensitivity for unbalanced rearrangements (35/40 detected duplications, 21/23 detected deletions) sized from 5 kb to 5000 kb and 84% sensitivity for balanced rearrangements (21/25 detected).

#### P16.83

#### Whole platelet transcriptome profiling confirms a high activation status in platelet components involved in transfusion reactions

C. Aloui<sup>1</sup>, J. Fagan<sup>2</sup>, F. Salin<sup>3</sup>, I. Lesur<sup>3,4</sup>, H. Banamara<sup>2</sup>, C. Mounier<sup>5</sup>, E. Tavernier<sup>5</sup>, F. Cognasse<sup>2,4</sup>, O. Garraud<sup>6,1</sup>, S. Laradi<sup>1,1</sup>,

<sup>1</sup>GIMAP - EA3064, Faculté de médecine, Université de Lyon, Saint Etienne, France,

<sup>2</sup>Etablissement Français du Sang Rhône-Alpes-Auvergne, Saint Etienne, France, <sup>3</sup>INRA, UMR 1202 Biodiversité Génèses et Ecosystèmes, Genome Transcriptome Facility, Cestas, France, <sup>4</sup>HelixVenture, Mérignac, France, <sup>5</sup>Institut de Cancérologie Lucien Neuwirth, Saint Priest en Jarez, France, <sup>6</sup>INTS - Institut National de la Transfusion Sanguine, Paris, France.

Introduction: Blood Platelets in stored blood components (PCs) for transfusion release panoply of molecules thought to be associated with transfusion adverse events (AEs). Successive measures taken overtime to minimize AEs proved to be successful but PC transfusion still leads to unwanted inflammatory reactions in a certain number of patients. The rationale of this study is to decipher the transcriptome of PCs involved in acute transfusion reactions (ATRs).

Methods: Total RNAs were extracted from 5 leukodepleted PCs implicated in ATR and 5 PC matched controls. We investigated the transcriptome using the Proton platform (Ion torrent, Life Technology). Data were mapped using CLC Bio software (Aarhus, Denmark). Transcript were counted using HTSeq-count software. Differentially expressed genes were identified using DESeq2 package (Bioconductor) and then underwent a functional enrichment analysis by PANTHER (www.Pantherdb.org).

Results: The analysis showed a highly complex transcriptional setting. The analysis of upregulated genes identified mainly two enriched pathways related to platelet activation (e.g. Integrin signaling pathway - P00034) and inflammatory process (e.g. chemokine signaling pathway - P00031). Functional analysis considering the Biological process identified proteins related to platelet metabolic and cytoskeletal rearrangement (e.g. GO: 0008152; GO: 0050896) as most enriched and then followed by apoptotic process (GO: 0006915).

Conclusion: Our results suggest that platelets are more activated in ATR inducing PCs. The activation of chemokine signaling pathway could explain findings of several single studies that identified biological response modifiers upregulated in PCs involved in ATR.

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#### P16.84

#### Integrated variant annotation and filtering using R/Bioconductor and the VariantFiltering package

D. M. Elurbe<sup>1</sup>, P. Puigdevall<sup>2</sup>, M. Milà<sup>3</sup>, R. Castelo<sup>2</sup>,

<sup>1</sup>Radboud University, Nijmegen, Netherlands, <sup>2</sup>Universitat Pompeu Fabra, Barcelona, Spain, <sup>3</sup>Hospital Clínic, Barcelona, Spain.

The steady decrease in DNA sequencing costs facilitates the adoption of whole-exome sequencing (WES) in clinical genetic testing settings. The identification of disease-causing non-synonymous coding rare variants from WES data is straightforward with currently available software and databases. However, the larger number of diseased individuals being genetically profiled using WES technology also increases the number of pathogenic variants that remain uncharacterized. This typically happens with variants that do not appear in curated databases and occur in non-coding regions, often having a reduced penetrance in the population. To approach the identification of such pathogenic variants, approaches based on the integration of multiple annotation sources and filtering strategies are needed. One of the software platforms which can potentially offer the required flexibility and interoperability for this goal is the R/Bioconductor project and its DNA variant analysis infrastructure. On top of this infrastructure, we have built the VariantFiltering package with the aim of facilitating the annotation and filtering of both, coding and non-coding genetic variants. The main features of this software are: 1. integration of multiple annotation sources tracing provenance; 2. programmatic filtering with multiple strategies for both coding and non-coding variants, such as inheritance model, protein damage potential, minor allele frequency, gene and nucleotide conservation, (cryptic) splice site strength, etc., which can be extended by the user; 3. minimization of end-user scripting tasks with an interactive shiny web app.

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**P16.85****ALLEXES: A variant reference data repository for clinical routine diagnostics**B. Liesfeld<sup>1</sup>, R. Abou Jamra<sup>2</sup>, S. Leye<sup>1</sup>, R. Ewald<sup>1</sup>;<sup>1</sup>Limbus Medical Technologies GmbH, Rostock, Germany, <sup>2</sup>Institut für Humangenetik, Universitätsklinikum Leipzig, Leipzig, Germany.

The ever increasing number of detected variants poses great challenge for routine diagnostics. Many attempts have been made to address this problem. Current approaches have several shortcomings: 1) genotypic and phenotypic information of patients is not captured comprehensively, 2) handling continuously changing variant information is not supported, and 3) manual data curation is required, which adds an unacceptable effort to the clinical routine. As a result, most data silos are either short-lived or contain outdated information.

We evaluate the ALLEXES system that integrates variant annotation and curation into automated clinical processes from VCF file to the medical report. A cohort study of whole exome cases from different institutions was processed. Aggregated genotypic and phenotypic information was shared in an automated fashion. ALLEXES is a regulated medical device which is compliant with harmonized international standards for medical device software and usability.

The time required to evaluate clinically relevant variants was reduced significantly compared to conventional annotation and reporting methods. Allele frequency and clinical information related to variants were available in near-time to all participants in the ALLEXES network. Confidentiality of patient data was maintained.

Existing and upcoming new regulation of genetic testing imposes increasingly strict rules on data handling. On the other hand, the overwhelming amount of variant data can only be tackled by leveraging information that is currently distributed across many isolated data silos. This study demonstrates that suitable technology is available to tackle the challenge of interpreting variants while being compliant with strict regulations regarding security and patient safety.

**P16.86****Benchmarking quality and performance of WES/WGS variant calling workflows from next-generation sequencing data**S. Laurie<sup>1,2</sup>, M. Fernandez-Callejo<sup>1,2</sup>, S. Marco-Sola<sup>1,2</sup>, J. Trott<sup>1,2</sup>, S. Heath<sup>1,2</sup>, S. Beltran<sup>1,2</sup>, <sup>1</sup>Centro Nacional de Análisis Genómico (CNAG-CRG), Center for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain.

A number of factors must be taken into account when establishing a variant calling pipeline for high-throughput processing of whole exome (WES) and whole genome (WGS) sequencing reads. These include accuracy, time required, RAM and CPU usage, and ease of implementation. We have tested combinations of two aligners (GEM3 and BWA-MEM), and 3 variant calling algorithms (GATK Haplotype Caller, SAMtools and FreeBayes) for both WES and WGS experiments on the HapMap sample NA12878. We used publicly available Illumina Platinum WGS reads, and WES libraries generated and sequenced in-house for NA12878 as input. Accuracy was determined through comparison with a gold standard set of variant calls published by NIST. In addition we recorded the amount of time required, and estimates of memory and CPU usage for each step of the workflow. In general we find a relatively small number of differences between the variants identified using each workflow, with concordance with calls in the NIST set generally in excess of 99% for SNVs and 98% for short indels. However, we find large differences in demands on computing resources between the algorithms, which affects time and cost of analyses. In addition we have observed cases where there is good evidence for a variant position that is not recorded within the NIST reference variants set, indicating that while a valuable resource, it is not perfect. It should also be noted that there remains a large part of the genome (15-25%) where it is unclear if current algorithms can successfully identify short-variants.

**P16.87****MutationInfo: A tool to quick retrieve genomic position from HGVS variants**

A. Kanterakis, E. Kartsaki, G. Potamias;

Foundation for Research and Technology Hellas, Heraklion, Greece.

Today one of the most prominent nomenclatures for reporting genomic variants is HGVS (<http://www.hgvs.org/mutnomen/>). According to this, variations are reported in alliance to their relative position in a reference sequence. This sequence can be a transcript, a chromosome, a database accession number, just to name few. The complexity of HGVS and the sub-

sequent non-adherence from researchers makes the unique genomic positional identification of a variant, a cumbersome task. The most known tool that performs this task is mutalyzer ([www.mutalyzer.nl](http://www.mutalyzer.nl)) that requires strict HGVS adherence and is able to identify only 43% of the variants reported in PAH, BRCA2 and HbVar databases. Here we present MutationInfo, a tool that performs various heuristics in order to perform the same task through less strict and fuzzy methods. These heuristics include (1) the lookup in databases like UCSC tables, Variant Effect Predictor, mutalyzer and LOVD, (2) the use of existing HGVS parsers like biocommon/hgvs, (3) correct for common HGVS mistakes and (4) perform BLAT alignment search of the fasta sequence in the reference assembly. We also report the significant increase of identified variants on public databases including pharmGKB. We conclude that this tool can improve the identification of already reported variants in sequencing studies and therefore contribute to the clinical application of genetics.

Availability: <https://github.com/kantale/MutationInfo>

**P16.88****A whole genome reference panel for South European Populations**M. Coccia<sup>1</sup>, M. Mezzavilla<sup>2</sup>, C. Barbieri<sup>3</sup>, C. Sala<sup>3</sup>, N. Soranzo<sup>4,5</sup>, D. Toniolo<sup>3</sup>, P. Gasparini<sup>2</sup>, <sup>1</sup>University of Trieste, Trieste, Italy, <sup>2</sup>Experimental Genetics Division, Sidra, Doha, Qatar, <sup>3</sup>San Raffaele Scientific Institute, Division of Genetics and Cell biology, Genetics of common disorders Unit, Milano, Italy, <sup>4</sup>The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom, <sup>5</sup>Department of Haematology, University of Cambridge, Cambridge, United Kingdom.

Imputation with large reference panels from whole genome sequenced DNAs is a common practice to improve variant coverage in a study sample, particularly for rare variants. Incorporating a large number of populations with different ancestries, as in 1000G, increases the accuracy of imputation but it was also demonstrated that adding haplotypes belonging to the same ancestry of the study sample will increase accuracy even more.

However in the current 1000G reference panel used for imputation there are few South European populations, which could hinder the imputation effort for populations close to the Mediterranean area.

To address this issue we generated low coverage WGS data of 926 samples belonging to 3 villages from Northern and Southern Italy participating to the INGI network. We describe here a reference panel of 1852 haplotypes composed by 20,829,813 SNPs and 2,490,202 INDELs selected after passing a strict QC: 43% of the selected sites are shared among all three Isolates, with 41 to 50% low frequency variants (MAF<1%) private to the INGI-cohorts with respect to the EUR samples from 1000G Phase 3.

We show that joining this panel with the standard 1000G reference, as well as with other references deriving from Northern European populations, increases imputation's accuracy of low frequency variants in Italian genotyped cohorts and possibly in other southern European cohorts: we noticed an 11% increment on average quality, using the merged INGI-1000G panel. This new resource will allow researchers to carry out imputation and GWAS for South European population in a more efficient way.

**P16.89****Two thousand Japanese whole genome reference panel in Japan and Bioinformatics**

M. Nagasaki, Y. Kawai, K. Kojima, T. Mimori, K. Misawa, T. Shibata, Y. Yamaguchi-Kabata, Y. Sato, R. Yamashita, N. Fuse, S. Ito, N. Nariai, Y. Kuroki, I. Danjoh, S. Saito, F. Katsuoka, J. Yasuda, M. Yamamoto, ToMMo Japanese Reference Panel Project; Tohoku University, Sendai, Miyagi, Japan.

Introduction: Tohoku University Tohoku Medical Megabank Organization (ToMMo) located at the north-eastern Japan is developing a biobank with 150,000 volunteers that combines medical and genome information during the process of rebuilding from the Great East Japan Earthquake on Mar/11/2011. One of the missions of ToMMo is to reveal a fine genetic architecture of Japanese population to tackle the further genome wide associate study analysis by combining the knowledge, which is daily accumulated in this project, e.g. questionnaire data, physiological data, medical treatment records and other omics data from serum, plasma and immortalized lymphocytes.

Materials and Methods: We have created the reference panel with one thousand samples to cover MAF > 0.5% variants including short insertion, deletion, and large structural variants in Japanese for constructing the Japanese whole-genome reference panel (1KJPN). Currently we are constructing the second reference panel with two thousand samples (2KJPN). In these panels, to minimize the biases caused by the different equipment, protocol and bioinformatics analysis, we performed whole genome sequencing of thousands samples with 30x high coverage using the HiSeq 2500 rapid run mode and analyzed by the same bioinformatics pipeline.

**Results and Conclusions:** We demonstrate our bioinformatics analyses, e.g. HLA-typing and CNVs, preliminary findings to 1KJPN and 2KJPN, and another effort to construct Japanese reference genome with thousands novel insertions in genome wide.

This work was supported (in part) by Tohoku Medical Megabank Project (Special Account for reconstruction from the Great East Japan Earthquake).

## P16.90

### KGSeq: a software platform for a comprehensive downstream analysis in large-scale whole genome sequencing studies

J. Li, Y. Q. Song, P. Sham, M. X. Li;

The University of Hong Kong, Hong Kong, Hong Kong.

With the development of next-generation sequencing (NGS) technology, the cost of sequencing has tremendously decreased, which promotes the application of many sequencing methods in genetic studies, disease diagnose and so on. Especially, the whole genome sequencing (WGS) is a very promising method for geneticists because it can provide the comprehensive information of genotypes in both coding and non-coding regions, common and rare variants. However, the Big Data problem emerged at the same time due to the large-scale dataset of WGS and currently no professional tool can solve it at all points. KGSeq is a software platform for comprehensive downstream analysis of whole-genome sequencing data. It is comprised of six functional modules: quality control, filtration, annotation, pathogenic prediction at variants, pathogenic prediction at genes and statistical tests, which can meet almost all researchers' demands for WGS data analysis. The original bit-block genotype storage mode can save 90% space and be much faster than existing tools. The gap-filled annotation rescue over 1000 exonic variants that are ignored by other tools. Moreover, KGSeq reads the compressed variant call format (VCF) file by blocked GNU Zip format in parallel and parses the byte input stream directly. A heuristic algorithm is also used to facilitate the extracting information from text files. Finally, we compared KGSeq with the existing tools by 1000 Genomes Project AFR panel, and KGSeq always provided a more reasonable annotation and had a higher consistency with dbSNP database.

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## P16.91

### Alteration of *Sus Scrofa* duodenum gene expression profile as response to a low dose of ZEA: extrapolation and analysis in the context of human health

C. Braicu<sup>1,2</sup>, R. Cojocneanu-Petric<sup>1</sup>, A. Jurj<sup>1</sup>, D. Marin<sup>3</sup>, I. Taranu<sup>3</sup>, I. Berindan-Neagoe<sup>1</sup>;

<sup>1</sup>Research Center of Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu Univ, Cluj-Napoca, Romania, <sup>2</sup>Department of Functional Genomics and Experimental Pathology, The Oncological Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania, <sup>3</sup>Laboratory of Animal Biology, National Institute for Research and Development for Biology and Animal Nutrition, Balotesti, Romania.

**Introduction:** Zearelenone (ZEA) is a mycotoxin produced by Fusarium species, initially capturing the attention for its function of estrogenic mimic role. But the mechanism of this mycotoxin remains unclear. Therefore we evaluated the effect of 100ppb ZEA on transcriptomic pattern at duodenum level, first physical and chemical barrier in animals and humans.

**Material and methods:** The alteration of gene expression pattern caused by ZEA exposure at *Sus scrofa* duodenum was done using a custom microarray design (AMADID: 056850, Agilent technology). Data were analyzed with Features Extraction and Gene Spring. Then the data were extrapolated to human orthologues and analyzed in the context of human health using Ingenuity Pathways Analysis (IPA). The validation was done for six genes by qRT-PCR and three cytokines by ELISA.

**Results:** We had 1576 upregulated transcripts and 2446 downregulated transcripts as response to ZEA exposure at duodenum level considering as cut-off value fold-change $\geq$ 2 and p-value $<0.05$ . The qRT-PCR and ELISA data confirmed the microarray results. The extrapolated human orthologues used for IPA analysis, revealing the activation of immune response, mitogen-activated-protein kinases, Toll-like receptors and carcinogenic pathway.

**Conclusions:** The effects of ZEA are complex at duodenum level, being connected not only with the alteration of the immune response but also the activation of the genes related to early colorectal carcinogenesis. The effects concerning the carcinogenic risk represent an important issue for future studies, particularly in the context of co-occurrence with other mycotoxins or environmental risks factors, targeting multiple genetic alterations.

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## P17 Epigenetics and Gene Regulation

### P17.01

#### DNA methylation and hydroxymethylation patterns in non-cultured human uterine leiomyoma cells

A. S. Koltsova<sup>1,2</sup>, A. A. Pendina<sup>1,2</sup>, O. A. Efimova<sup>1,2</sup>, A. V. Tikhonov<sup>1,2</sup>, N. S. Osinovskaya<sup>2</sup>, O. V. Malysheva<sup>2</sup>, N. Y. Shved<sup>2</sup>, T. V. Kuznetsova<sup>1,2</sup>, V. S. Baranov<sup>1,2</sup>;

<sup>1</sup>Saint Petersburg State University, St. Petersburg, Russian Federation, <sup>2</sup>D.O.Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation.

We have studied DNA methylation and hydroxymethylation patterns in cells from human histologically confirmed uterine leiomyomas (ULs). We performed our study in non-cultured cells because culture conditions may alter hydroxymethylation pattern. UL samples were obtained by surgery, suspended in collagenase type IV solution, then treated with hypotonic solution (0.9% sodium citrate + 0.55% potassium chloride) and fixed on slides with methanol:acetic acid, 1:1. 5-methylcytosine and 5-hydroxymethylcytosine were detected by indirect immunofluorescence.

We compared hydroxymethylation patterns between ULs positive and negative for MED12 mutation, as well as between ULs excised in follicular and lutein phase. In all 22 ULs, we detected 3 types of nuclei: strongly hydroxymethylated, averagely hydroxymethylated, and non-hydroxymethylated. Hydroxymethylation level was estimated visually by fluorescence intensity and then assessed in ImageJ. When comparing hydroxymethylation patterns between ULs positive and negative for MED12 mutation, no significant difference was detected (Unpaired t-test with Welch's correction, P=0.7854). When comparing hydroxymethylation patterns between ULs excised in different menstrual phases, we found that 5-hydroxymethylcytosine level was significantly higher in ULs excised in follicular phase. We have shown a negative correlation between the 5-hydroxymethylcytosine level and the day of menstrual cycle at the time of UL excision (Spearman correlation coefficient=-0.473, P<0.05). DNA methylation was detected in all nuclei of all 22 ULs and difference among nuclei was not pronounced.

Our results suggest that genome activity in ULs, determined by their hydroxymethylation pattern, varies during menstrual cycle.

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### P17.02

#### ADP-ribosylation of distal cis-regulatory chromatin regions of proinflammatory cytokines as a mechanism, which determines their transcription in proinflammatory macrophages M1

E. Wiśniki, A. Robaszekiewicz;

Department of Environmental Pollution Biophysics, Institute of Biophysics, University of Lodz, Lodz, Poland.

**Introduction:** Proinflammatory macrophages M1 derived from are responsible for the secretion of proinflammatory factors such as cytokines attracting other immune cells to accumulate in the site of the inflammation. As the differentiation and polarization of macrophages is followed by extensive chromatin remodeling we checked whether transcription of cytokines is solely regulated by NF- $\kappa$ B (p50/p65) binding to proximal cis-regulatory elements or involves enhancers and epigenetic events such as ADP-ribosylation carried out by PARP1 within these regions.

**Materials and Methods:** Human monocytes isolated from peripheral blood were differentiated and polarized with GM-CSF, while human monocyte cell line THP1 with PMA. Expression of macrophage markers and cytokines was analyzed with real-time PCR, association of NF- $\kappa$ B with chromatin and epigenetic modifications with ChIP, gene silencing with siRNA/shRNA. The interaction of chromatin proximal and distal regulatory elements was studied with 3C method.

**Results:** The differentiation of macrophages is accompanied by activation of distal regulatory elements and their ADP-ribosylation inhibition of which enhances NF- $\kappa$ B (p50/p65) binding to chromatin and expression of cytokines. The silencing of lineage determining factor - PU.1, which enables NF- $\kappa$ B (p50/p65) interaction with enhancers restores the monocyte-like gene expression. Moreover, inhibition of ADP-ribosylation as well as PARP1 silencing facilitates the chromatin looping resulting from enhancer interaction with cytokine promoters.

**Conclusions:** ADP-ribosylation of chromatin distal regulatory elements, undergoing activation during terminal macrophage differentiation, regulates the interaction of NF- $\kappa$ B with these regions.

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## P17.03

## Investigating the involvement of long non coding RNA in ALS

S. Gagliardi<sup>1,2</sup>, S. Zucca<sup>1,2</sup>, O. Pansarasa<sup>1</sup>, L. Diamanti<sup>3</sup>, M. Bondoni<sup>1,4</sup>, M. Valente<sup>1</sup>, M. Ceroni<sup>2</sup>, C. Cereda<sup>1</sup>;

<sup>1</sup>Center of Genomics and post-Genomics, C. Mondino National Institute of Neurology Foundation, Pavia, Italy, <sup>2</sup>Laboratory of Bioinformatics, Mathematical Modelling and Synthetic Biology, Department of Electrical, Computer and Biomedical engineering, University of Pavia, Pavia, Italy, <sup>3</sup>Neurology Department, IRCCS National Neurological Institute C. Mondino, University of Pavia, Pavia, Italy, <sup>4</sup>Department of Brain and behavioral science, University of Pavia, Pavia, Italy.

**Introduction:** The importance of various classes of regulatory non-coding RNAs (ncRNAs) in diseases is increasingly being recognized. We propose to perform a systematically profile, by RNA-Seq approaches, of the lncRNAs and mRNAs in human ALS lymphocytes mutated, unmutated and controls with the aim of extending our knowledge on molecular alterations of transcriptome and obtaining new data about its regulation.

**Materials and Methods:** three cohort of ALS mutated patients (FUS, SOD1 and TARDBP) have been recruited and have been compared with healthy subjects and ALS sporadic (non mutated) patients. RNA libraries have been prepared by TruSeq Stranded Total RNA with Ribo-Zero Gold kit (illumina). **Results:** 28 common genes were found differentially expressed in all groups compared to controls. About lncRNA, the data showed that in SOD1 group 32 linc were found differentially expressed, 18 in TARDBP, 5 in FUS and 8 in SALS group respectively.

**Discussion:** Whole transcriptome analysis showed a general down-regulation in genes expression in all the studied groups. Moreover our preliminary data showed a comparable regulation between two groups positive for mutations in TARDBP and FUS, both involved in the same pathways, while a different profile arises from SOD1 and SALS groups. About lncRNAs the 56 lincsRNAs are down-modulated by different extent in healthy donors and one SALS and one SOD1 sample. On the other side they are mainly up-modulated in the other disease samples. This preliminary analysis seems to indicate that it is not possible, with this set of lincsRNAs, to discriminate between the different mutation states.

## P17.05

## Interethnic DNA methylation difference and its implications of cancer drug response

S. Chu<sup>1,2,3</sup>, H. Yang<sup>1,2</sup>;

<sup>1</sup>Institute of Statistical Science, Academia Sinica, Taipei, Taiwan, <sup>2</sup>Bioinformatics Program, Taiwan International Graduate Program, Institute of Information Science, Academia Sinica, Taipei, Taiwan, <sup>3</sup>Institute of Biomedical Informatics, National Yang-Ming University, Taipei, Taiwan.

**Introduction:** Using DNA methylation variants for predicting drug response represents an important application in pharmacogenetics. However, how these variants are influenced by the ethnic difference is still not studied systematically.

**Materials and Methods:** We collected DNA methylation and gene expression data of primary tumor tissues of four cancer types (breast, colon, head and neck, and uterine corpus) from The Cancer Genome Atlas and lymphoblastoid cell lines from The International HapMap Project for African and European ancestry populations. The methylation data were generated by the Infinium HumanMethylation450 BeadChip and gene expression data were generated by RNA sequencing experiments. After data quality control, regression models with batch effect adjustments were used to examine the association between CpG sites and ethnic population as well as to investigate the relationship between differential methylation and gene expression. **Results:** We identified 264 CpG sites in drug response genes significantly associated with ethnicity. Among them, differential methylation in 19 CpG sites was highly related to the differential expression of the corresponding genes (e.g., *SLC7A5* gene expression and its intronic CpG site cg27560818). A further comparison of the ethnicity associated CpG sites in cancers and HapMap datasets revealed significant methylation differences in both of global distributions and individual CpG sites.

**Conclusions:** Our results demonstrate ethnic effects on pharmacogenetics and the patterns may change by tissue types. These findings provide useful information for pharmacogenetic prediction in clinical practices and future pharmacogenetic research.

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## P17.06

## Aberrant promoter methylation of matrix and transmembrane proteins encoding genes in breast cancer

O. A. Simonova<sup>1</sup>, E. B. Kuznetsova<sup>1,2</sup>, E. V. Poddubskaya<sup>3</sup>, R. A. Kerimov<sup>3</sup>, V. V. Rudenko<sup>1</sup>, A. S. Tanas<sup>1,4</sup>, V. V. Strelnikov<sup>1,4</sup>, D. V. Zaletaev<sup>1,2</sup>;

<sup>1</sup>Research Centre for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>Sechenov First Moscow State Medical University, Moscow, Russian Federation, <sup>3</sup>Blokhin Russian Research Center for Oncology (FSBI N.N.Blokhin), Moscow, Russian Federation, <sup>4</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

**Introduction:** Matrix and transmembrane proteins play fundamental role in cell's live regulation. They are involved in maintenance of tissue architecture, signals transduction, wound healing, adhesion and play significant role in tumor development.

**Materials and methods:** We evaluated epigenetic regulation of 12 laminin-encoding genes (*LAMA1*, *LAMA2*, *LAMA3A*, *LAMA3B*, *LAMA4*, *LAMA5*, *LAMB1*, *LAMB2*, *LAMB3*, *LAMC1*, *LAMC2*, *LAMC3*), 8 integrins (*ITGA1*, *ITGA2*, *ITGA3*, *ITGA4*, *ITGA6*, *ITGA7*, *ITGA9*, *ITGB1*), 2 nidogens (*NID1*, *NID2*), the dystroglycan gene *DAG1* and 10 matrix metalloproteinases-encoding genes (*MMP2*, *MMP14*, *MMP15*, *MMP16*, *MMP17*, *MMP21*, *MMP23B*, *MMP24*, *MMP25*, *MMP28*) and 4 genes of tissue inhibitors of metalloproteinases (*TIMP1*, *TIMP2*, *TIMP3*, *TIMP4*) in 186 samples of breast cancer, 186 paired adjacent nonmalignant samples and 6 samples of normal mammary gland from autopsy by methylation sensitive PCR and bisulfite sequencing.

**Results:** Promoters of the *LAMA3A*, *LAMB2*, *LAMB3*, *LAMC2*, *MMP14*, *MMP21*, *TIMP1*, *TIMP4* genes were constitutively methylated in breast tissues. Promoters of 15 genes have demonstrated abnormal methylation in BC (table). In small number of cases genes *LAMA1*, *LAMA2*, *LAMB1*, *NID1*, *ITGA4*, *ITGA9* were methylated not only in BC, but also in paired adjacent nonmalignant samples.

**Conclusions:** Complex alteration of the studied genes methylation can be important for understanding of the dramatic changes in tissue architecture and signal transduction during tumor growth and development.

This study was funded by RFBR, research project 14-04-01792.

Gene	Methylation in breast cancer (%)	Methylation in normal mammary gland from autopsy (%)	Association with clinicopathological features
LAMA1	29,4 (50/170)	0	-
LAMA2	25,8 (48/186)	0	Her2+
LAMB1	28,5 (51/179)	0	Her2+
LAMC1	3,7 (7/186)	0	-
ITGA1	13,3 (20/150)	0	Ductal cancer type
ITGA4	29,3 (44/150)	0	Tumor size T3-T4
ITGA7	3,3 (5/150)	0	Her2+
ITGA9	40,6 (61/150)	0	ER+
NID1	37,3 (56/150)	0	Her2+
NID2	39,3 (59/150)	0	-
MMP2	7,95 (14/176)	0	-
MMP23B	17,24 (30/174)	0	Lack of ER expression Lack of PR expression
MMP24	10 (15/150)	0	Her2+
MMP25	20,33 (24/118)	0	-
MMP28	5,14 (9/175)	0	Metastasis M1 Stage 2A

## P17.07

## Capture Hi-C identifies compelling candidate causal genes and enhancers for multiple sclerosis in the 6q23 region

P. Martin<sup>1</sup>, A. McGovern<sup>1</sup>, K. Duffus<sup>1</sup>, A. Yarwood<sup>1</sup>, S. Schoenfelder<sup>2</sup>, N. J. Cooper<sup>3</sup>, A. Barton<sup>1,4</sup>, C. Wallace<sup>3,5</sup>, P. Fraser<sup>2</sup>, J. Worthington<sup>1,4</sup>, S. Eyre<sup>1</sup>, G. Orozco<sup>1</sup>;

<sup>1</sup>Arthritis Research UK Centre for Genetics and Genomics, Centre for Musculoskeletal Research, Institute of Inflammation and Repair, Faculty of Medical and Human Sciences, Manchester Academic Health Science Centre, The University of Manchester, Manchester, United Kingdom, <sup>2</sup>Nuclear Dynamics Programme, The Babraham Institute, Cambridge, United Kingdom, <sup>3</sup>JDRF/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, NIHR Cambridge Biomedical Research Centre, Cambridge Institute for Medical Research, University of Cambridge, Wellcome Trust/MRC Building, Cambridge, United Kingdom, <sup>4</sup>NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester Foundation Trust, Manchester Academic Health Science Centre, Oxford Road, Manchester, United Kingdom, <sup>5</sup>MRC Biostatistics Unit, Cambridge Institute of Public Health, Forvie Site, Robinson Way, Cambridge Biomedical Campus, Cambridge, United Kingdom.

**Introduction:** Genome wide association studies (GWAS) have been successful in identifying variants associated with complex disease, including multiple sclerosis (MS). Many of these variants lie in enhancer regions, although it is often unclear which gene they affect. Capture Hi-C (CHi-C) can be used to study long-range interactions at high resolution between enhancers and target genes. The 6q23 region is a pan-autoimmune locus associated with multiple autoimmune diseases. Our aim was to identify MS causal genes in the 6q23 region by studying chromatin interactions involving MS associated variants and further refining the causal variants.

**Methods:** Interactions with MS associated regions in the 6q23 locus, defined

as SNPs in  $r^2 \geq 0.8$  with the lead association, were identified by CHi-C. SNP causality was further refined using HaploReg, RegulomeDB and eQTL data. Results: Complex long-range interactions were observed between MS associated regions and eight gene promoters, as well as between each other. These interactions implicate several genes including *AHI1*, *SGK1*, *BCLAF1*, *IL20RA*, *IL22RA2*, *IFNGR1* and *TNFAIP3*, some representing compelling autoimmune and MS candidates. Bioinformatics analysis of SNPs involved in these interactions further refined the SNPs to enhancer regions and identified many of the target genes as actively transcribed regions. Conclusions: This investigation has identified many compelling genes which could be involved in MS pathogenesis. Additionally, it has refined the associations further, in some cases to specific enhancer regions which will require further functional validation. This work has the potential to provide novel therapies or drug repositioning and improving patient outcome.

#### P17.08

##### Alanine expansions associated with Congenital Central Hypoventilation Syndrome (CCHS) impair PHOX2B homeodomain-mediated dimerisation and nuclear import

*D. Belperio<sup>1</sup>, S. Di Lascio<sup>1</sup>, R. Benfante<sup>2,1</sup>, D. Fornasari<sup>1,2</sup>,*

<sup>1</sup>University of Milan, Milan, Italy, <sup>2</sup>CNR-Neuroscience Institute, Milan, Italy.

Introduction: Polyalanine expansions in the 20-residues region of the human PHOX2B gene, a key regulator of autonomic nervous system development, lead to Congenital Central Hypoventilation Syndrome, a neurodevelopmental disorder characterised by a failure in the autonomic control of breathing. Elongation of the alanine stretch leads to a protein with altered DNA-binding, transcriptional activity, nuclear localisation, and cytoplasmic aggregates formation. Previous studies support the idea that PHOX2B mutant proteins display both loss and new toxic gain of function, resulting in a dominant-negative effect. As PHOX2B forms homodimers and heterodimers with its parologue PHOX2A in vitro, we tested the hypothesis that the dominant-negative effect of the mutated proteins are due to non-functional interactions with the wild-type protein or PHOX2A. Moreover we investigated the effects of the longest polyalanine expansions on the homeodomain-mediated nuclear import.

Material and methods: To study hetero- and homo-dimerisation we used co-immunoprecipitation assay, and mammalian two-hybrid system. Deletion analysis and immunocytochemistry were used to study the consequences of alanine expansion on the sub-cellular localisation.

Results and conclusions: Our findings show that PHOX2B forms homodimers, heterodimerise weakly with mutated proteins, exclude the direct involvement of the polyalanine tract in dimer formation, and indicate that mutated proteins retain partial ability to form heterodimers with PHOX2A, with a possible role in the pathogenic process. Moreover our data show that the expanded C-terminus interferes with nuclear import process. These results provide novel insights into the effects of the alanine tract expansion on PHOX2B folding and activity.

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#### P17.09

##### Newly identified PHOX2B target genes as drug targets in Congenital Central Hypoventilation Syndrome (CCHS)

*S. Cardani<sup>1</sup>, D. Belperio<sup>1</sup>, S. Di Lascio<sup>1</sup>, R. Benfante<sup>2,1</sup>, D. Fornasari<sup>1,2</sup>,*

<sup>1</sup>University of Milan, Milan, Italy, <sup>2</sup>CNR - Neuroscience Institute, Milan, Italy.

Introduction: Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880) is a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control. Frameshift mutations (5%) and polyalanine triplet expansions (95%) have been detected in the coding region of the transcription factor PHOX2B, responsible for the proper development and function of the autonomic nervous system. Consistent with its role as transcriptional regulator, it is reasonable to suppose that transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. Current research on treatments of CCHS is focused on counteracting the toxic effects of the mutated PHOX2B protein, and stemming from the fortuitous observation that progestin Desogestrel can relieve some symptoms of the disease, by a not yet identified molecular mechanism, lead us to identify new PHOX2B target genes as potential pharmacological targets for alternative molecules without contraceptive effects.

Methods: Identification of PHOX2B regulated genes has been carried-out by ChIP-seq analysis in IMR32 cells. The expression of PHOX2B target genes will be selectively validated by comparing wild-type and CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells.

Results and conclusion: Gene Ontology analysis of the set of peak-associated genes identified several enriched terms in categories consistent with PHO-

X2B role during autonomic nervous system development and maintenance. Further, we show that Desogestrel enhanced the expression of some relevant PHOX2B target genes in a promoter specific manner, by acting on the activity of the wild type as well as mutant protein.

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#### P17.10

##### Identification of key regulator elements in acute celiac response through whole genome coexpression analysis

*I. Romero-Garmendia<sup>1</sup>, K. Garcia-Etxebarria<sup>1</sup>, H. Hernandez-Vargas<sup>2</sup>, A. Jauregi-Miguel<sup>1</sup>, L. Plaza-Izurieta<sup>1</sup>, M. Legarda<sup>3</sup>, I. Iraizozza<sup>3</sup>, Z. Herceg<sup>2</sup>, N. Fernandez-Jimenez<sup>1,2</sup>, J. Bilbao<sup>1</sup>,*

<sup>1</sup>Immunogenetics Research Laboratory, Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country-UPV/EHU and BioCruces Health Research Institute, Leioa, Spain, <sup>2</sup>Epigenetics Group, International Agency for Research on Cancer (IARC), Lyon, France, <sup>3</sup>Pediatric Gastroenterology Unit, Cruces University Hospital (UPV/EHU), Barakaldo, Spain.

Introduction: In celiac disease (CD) gliadin provokes a coordinated response and the disruption of the coexpression in gene networks. The aim of this study was to analyze coexpression in the whole genome under acute gliadin exposure, and to identify regulator elements that could underlie these alterations in the context of CD.

Materials and Methods: We identified differentially coexpressed genes in an expression microarray from gluten-free diet (GFD) CD biopsies cultured with/out gliadin. We performed miRNA and transcription factor (TF) enrichment analyses and selection of candidate regulators. Expression of candidates was measured in clinical samples and the activation of one of the TFs (IRF1) was further characterized in C2BBe1 cells upon gliadin challenge. Binding of IRF1 to the *in silico*-predicted targets was measured by chromatin immunoprecipitation (ChIP).

Results: Two miRNAs and 3 TFs were selected as final candidates. One miRNA was underexpressed while 2/3 TFs were overexpressed in active disease. IRF1 was overexpressed at a protein level in the nuclear fraction of the C2BBe1 cells after gliadin exposure, although we did not see notable differences in the immunofluorescence assay. Finally, the IRF1 ChIP experiment performed in cells showed altered binding upon gliadin exposure in genes that were differentially coexpressed/expressed in acute CD.

Conclusions: Gliadin alters coexpression in CD. Moreover, our pipeline was able to identify regulators that could be relevant to disease. Particularly, IRF1 showed differential expression in patients and altered binding to several targets upon gliadin challenge in C2BBe1 cells, suggesting a general effect of gluten in physiological conditions.

#### P17.11

##### GC-rich DNA activates NFkB signaling pathway in different types of human cells via TLR9 receptor

*V. Sergeeva<sup>1</sup>, S. Kostyuk<sup>1</sup>, E. Ershova<sup>1</sup>, L. Kameneva<sup>1</sup>, E. Malinovskaya<sup>1</sup>, N. Veiko<sup>1</sup>, N. Mordkovich<sup>2</sup>, V. Veiko<sup>2</sup>, N. Okorokova<sup>2</sup>,*

<sup>1</sup>FSBI "Research Centre For Medical Genetics", Moscow, Russian Federation, <sup>2</sup>Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russian Federation.

Introduction: It has been established that cell-free DNA circulates throughout the bloodstream, affecting cells. Diseases can cause GC-enrichment of cfDNA pool (cerebral atherosclerosis, heart attack, rheumatic arthritis, cancer). Our aim was to investigate the exact mechanism of cfDNA-cell interaction.

Materials and methods: GC-rich DNA fragments were obtained by inserting the CpG rich fragment of rDNA in a vector (pBR322). mRNA was isolated from different types of human cells (MSCs, HUVECs, PBMCs, HELFs) using an RNeasy Mini kit (Qiagen, Germany). Gene expression level was assessed using RT-PCR (TBP and GAPDH as internal standards). Statistics was performed using Statgraphics software.

Results: GC-rich DNA plasmid (50 ng/ml, 3h incubation) increases TLR9 and its adapter MyD88 level in human MSCs, HUVECs, PBMCs and HELFs (TLR9 3-; 2,5-; 2,8-; 3,7-fold and MyD88 3-; 2,5-; 2,8-; 3,7- fold, respectively). GC-rich DNA plasmid activates NFkB signaling pathway in these types of cells, NFkB translocates to the nucleus and genes of NFkB signaling pathway: MAP3K1, MAP4K4, NFKB1A, REL, IKBKB, RelA (p65), NFRKB, NFKB1 and NFKB2 increase 2 - 5,5-fold. Expression of NFkB target genes TNF, IL1B, IL8, IL6, TNFRSF1A increase 2 - 4-fold in all types of cells. Combination of chloroquine with GC-rich DNA neither leads to the increment in MyD88 level nor activates NFkB signaling pathway. The vector itself doesn't affect TLR9 pathway.

Conclusion: GC-rich DNA increases expression of transcription factor gene NFkB and its target genes in MSCs, HUVECs, PBMCs and HELFs via TLR9 receptor.

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## P17.12

### Hypomethylation of long interspersed nuclear element-1 (LINE-1) in T-lymphocytes from patients with CF

E. Kvaratskhelia<sup>1,2</sup>, N. Daburashvili<sup>1</sup>, E. Maisuradze<sup>1</sup>, M. Gagua<sup>1</sup>, L. Margvelashvili<sup>3</sup>, E. Abzianidze<sup>2</sup>;

<sup>1</sup>Tbilisi State Medical University, Institute of Medical Biotechnology, Tbilisi, Georgia,

<sup>2</sup>Tbilisi State Medical University, department of Molecular and Medical Genetics, Tbilisi, Georgia, <sup>3</sup>Hospital „New Life“, Department of Medical genetics and Diagnostics, Tbilisi, Georgia.

**BACKGROUND:** T-lymphocytes from patients with CF are characterized by reduced expression of anti-inflammatory cytokines and elevated levels of pro-inflammatory cytokines. Although mechanisms responsible for cytokine dysregulation is not entirely clear epigenetic mechanisms such as DNA Methylation might contribute. Previous studies have identified abnormal DNMTs level in T-Lymphocytes from CF patients which was correlated with amount of IL-10. In this study we examined the methylation levels of genomic DNA (5-mC content) and long interspersed nuclear element 1s (LINE-1) in CD4+ T - Lymphocytes derived from CF individuals and healthy subjects. **METHODS:** This study was approved by the ethic committee of TSMU. Peripheral blood was obtained by venepuncture from CF and healthy subjects. CD4+ T cells were isolated from PBMC using the CD4+ T Cell Isolation Kit (Miltenyi Biotec GmbH). Genomic DNA was extracted using Quick-DNA Universal Kit (Zymo Research) and Global DNA methylation was measured using the Methylated DNA Quantification Kit (abcam). Methylation levels of LINE-1 in normal and CF CD4+ T Lymphocytes were examined by the combined bisulfite restriction analysis- long interspersed nuclear element 1s (COBRA-LINE1).

**RESULTS:** Global DNA methylation was significantly reduced in CF CD4+ T cells compared with controls. In addition, hypomethylation of LINE-1 was prominent and the methylation level of LINE-1 was correlated with global genomic 5-methylcytosine content in CF CD4+ T lymphocytes.

**CONCLUSIONS:** Abnormal DNA methylation especially hypomethylation of interspersed repetitive sequences can have a crucial effect on gene expression resulting in aberrant immune responses in CF CD4+ T cells.

## P17.13

### Dasatinib regulates lncRNA expressions in chronic myeloid leukemia

C. Kayabasi, S. Yilmaz Süslüer, T. Balci, B. Özmen Yelken, Z. Mutlu, C. Çalışkan Kurt, B. Gökber Bağca, Ç. Biray Avcı, F. Şahin, G. Saydam, C. Gündüz; Ege University, Izmir, Turkey.

Dasatinib is an ATP-competitive tyrosine kinase inhibitor used for the treatment of Chronic Myeloid Leukemia (CML). Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that play pivotal roles in modulating the cancer epigenome through regulating gene expression by variety of mechanisms including transcription, posttranscriptional processing, chromatin modification, genomic imprinting. We aimed to determine the changes of lncRNA expression profile in consequence of dasatinib treatment.

Cytotoxic and apoptotic effects of dasatinib on K562 cells was evaluated with WST1 and Apo-DIRECT with flow cytometry, respectively. LncRNA expression levels were examined with real-time RT-qPCR.

IC50 dose of dasatinib was calculated as 23.47 μM. Dasatinib induced apoptosis 5.32 fold compared to untreated control cells. Dasatinib upregulated H19 that plays role in epigenetic regulation through DNA methylation 16.44 fold and tumor suppressor RMST 16.2 fold. ANRIL that suppresses the expression of DNA damage response genes INK4a, INK4b, ARF, was downregulated 12.64 fold with dasatinib treatment. Dasatinib down-regulated poor prognostic biomarker HOTAIR 3.83 fold, HOTAIRM that is cell cycle progression regulator during myeloid maturation 6.01 fold and oncogenic MIAT 9.12 fold. Interestingly, members of prostate specific regulator of cell proliferation are all suppressed with dasatinib treatment in CML cells (PCAT43, PCAT1, PCAT32, PCAT29 down-regulated 13.29, 9.04, 8.9, 7.56 fold, respectively).

These novel findings showed that dasatinib has significant effects on lncRNA expression levels which are associated with tumorigenesis. Further researches for determining the target genes of these lncRNAs is essential in order to clinical understanding of the CML molecular pathogenesis and new treatment strategies.

## P17.14

### Hsa-miR-451 expression in white blood cells of CML patients:

### differential expression in optimal imatinib responders and non-responders

I. Soltani, K. Douzi, H. Gharbi, I. Ben hassine, M. Teber, S. Abbes, S. Menif; Pasteur Institute Tunisia, Tunis, Tunisia.

**Introduction:** The treatment of chronic myeloid leukemia (CML) has been greatly revolutionized by the development of imatinib mesylate (IM). Despite high efficiency of IM, acquired resistance has been observed in a significant proportion of patients. Aside mutations of the BCR/ABL gene, downstream factors may contribute to treatment failure. Accumulating evidence for aberrant expression of miR-451 in several types of human cancer suggests that it may play a critical role in treatment response to IM in patients with CML.

**Materials and Methods:** 29 responders (molecular remission) and 30 non-responders were included in this study. Both miR-451 expression and MYC transcript expression were measured by real-time quantitative RT-PCR assay. Analysis of relative expression of miR-451 and MYC between groups was calculated according to the 2-ΔCt method. Putative targets of miR-451 were predicted by TargetScan and Microcosm. In addition, we used Pach algorithm to identify transcription factor binding sites (TFBSs) in promoter region sequences of miR-451.

**Results:** Down-regulation of the expression of miR-451 was observed in non-responders as compared to responders. Furthermore, in silico analyses identified MYC as a potential target of miR-451 which in turn may regulates miR-451 expression as a transcription factor. Increased level of MYC was also detected in resistant patients when compared to optimal imatinib responders. Combination of these findings suggests that there may exist a feedback loop between miR-451 and MYC.

**Conclusions:** Our findings suggest that miR-451/MYC mini-circuitry may act as a potential therapeutic target and disruption of suggested regulatory loop could help to improve CML therapy.

## P17.16

### Methylation analysis of DNA repair genes in peripheral blood of Alzheimer's disease patients and healthy controls

A. Stoccoro<sup>1,2</sup>, P. Tannorella<sup>1</sup>, L. Chico<sup>3</sup>, G. Siciliano<sup>3</sup>, U. Bonuccelli<sup>2</sup>, F. Coppede<sup>1</sup>, L. Migliore<sup>1</sup>;

<sup>1</sup>Department of Translational Research and New Technologies in Medicine and Surgery, Section of Medical Genetics, University of Pisa, Pisa, Italy; <sup>2</sup>Doctoral School in Genetics Oncology and Clinical Medicine, Department of Medical Biotechnologies, University of Siena, Siena, Italy; <sup>3</sup>Department of Clinical and Experimental Medicine, University of Pisa, Neurological Clinic, Pisa, Italy.

**Introduction:** Several hypotheses have been formulated and tested over the last few years in order to explain the observed impairment in DNA repair activities in Alzheimer's disease (AD) neurons and peripheral tissues, including those involving the influence of gene polymorphisms, without reaching conclusive results. We hypothesized that this could partially result from epigenetic modifications of DNA repair genes, resulting in altered gene expression in those tissues. In the present study we searched for gene specific DNA methylation levels of a panel of DNA repair genes in peripheral blood cells of AD and healthy controls.

**Materials and Methods:** In a first step blood DNA of a subgroup composed by late-onset AD (LOAD) patients and healthy matched controls was screened with a commercially DNA methylation array able to evaluate the methylation levels of a panel of 22 genes involved in major DNA repair pathways. We then applied the cost-effective PCR based methylation sensitive-high resolution melting technique (MS-HRM), in order to evaluate the promoter methylation levels of some of the major DNA repair genes, namely *OGG1*, *PARP1*, *MRE11A*, *BRCA1*, *MLH1*, and *MGMT* in blood DNA from a wider cohort.

**Results:** Both approaches showed that all the investigated genes were largely hypomethylated in LOAD and control blood DNA, and no difference between groups was observed.

**Conclusions:** In summary, the present investigation suggests that there is no impairment in promoter methylation of the investigated DNA repair genes in blood DNA of AD patients.

**P17.17****Differential DNA methylation and comparison between boys and girls with ADHD**

*T. V. M. M. Costa<sup>1</sup>, F. A. Marchi<sup>2</sup>, C. Milani<sup>1</sup>, J. G. Damasceno<sup>1</sup>, E. A. Zanardo<sup>1</sup>, G. M. Novo-Filho<sup>1</sup>, M. M. Montenegro<sup>1</sup>, F. A. R. Madia<sup>1</sup>, A. T. Dias<sup>1</sup>, F. B. Piazzon<sup>1</sup>, R. L. Dutra<sup>1</sup>, A. M. Nascimento<sup>1</sup>, M. Rocha<sup>3</sup>, C. A. Kim<sup>4</sup>, V. Schuch<sup>5</sup>, C. B. Mello<sup>5</sup>, M. Muszkat<sup>5</sup>, L. D. Kulikowski<sup>1,2,4</sup>;*

<sup>1</sup>Faculdade de Medicina da USP, São Paulo, Brazil, <sup>2</sup>Laboratório NeoGene, AC Camargo Cancer Center, São Paulo, Brazil, <sup>3</sup>Centro de Reprodução Humana e Genética, Faculdade de Medicina do ABC, Santo André, Brazil, <sup>4</sup>Unidade de Genética, Departamento de Pediatria, Instituto da Criança, São Paulo, Brazil, <sup>5</sup>Núcleo de Atendimento Neuropsicológico Infantil Interdisciplinar (NANI), Departamento de Psicobiologia da Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil.

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorder diagnosed in childhood. ADHD is etiologically heterogeneous and has high heritability (around 80%). However, the genetic architecture of ADHD is still largely unknown. DNA methylation is the most stable epigenetic mark and is related to gene silencing. Therefore, the establishment of methylation profile in ADHD patients is important to guide the research key epigenetic factors in this disorder. DNA methylation profile was performed using DNA extracted from blood lymphocytes of 13 ADHD patients (9 boys and 4 girls, ages 06-14) by Illumina Infinium HumanMethylation450 BeadChip. We analyzed the array data using specific packages within the environment R and used the BRB-ArrayTools to make the cluster. We performed two analyzes: a comparison between boys and girls and a differential analysis of all the samples.

In the comparison between boys and girls we obtained 249 probes with  $\Delta\beta +0.20$ . In the differential analysis of all the samples, we selected 3,348 probes using  $SD > 0.1$  as selection criteria. We used the  $\beta$  values these 3,348 selected probes to make the samples cluster, which showed a strong separation of patients into two groups: the first formed of 4 girls and the second formed of 9 boys.

Thus, we suggested that it is quite probable that differences in DNA methylation profiles of boys and girls may be associated with the genetic basis of ADHD.

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**P17.18****Differential DNA methylation related to HDL functionality: an epigenome-wide approach**

*S. Sayols-Baixeras<sup>1,2</sup>, Á. Hernández<sup>1,3</sup>, I. Subirana<sup>4,1</sup>, C. Lluís-Ganella<sup>4</sup>, M. Fitó<sup>1,3</sup>, J. Marrugat<sup>4</sup>, R. Elosua<sup>1</sup>;*

<sup>1</sup>IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain, <sup>2</sup>Universitat Pompeu Fabra (UPF), Barcelona, Spain, <sup>3</sup>(CIBERONB) CIBER de Fisiopatología de la Nutrición y la Obesidad, Madrid, Spain, <sup>4</sup>CIBER Epidemiología y Salud Pública, Madrid, Spain.

Background: Cholesterol level in high-density lipoproteins (HDL-c) is a powerful predictor of cardiovascular risk. However, Mendelian randomization experiments and clinical trials do not support a causal association between HDL-c levels and cardiovascular disease. Therefore, the study of HDL biological functionality has become a hot topic in cardiovascular research. Epigenetics in general and DNA methylation in particular, are also associated with cardiovascular traits. However, there is a lack of data of the relationship between methylation and HDL functionality.

Aim: To assess the association between the two main HDL functions (cholesterol efflux capacity and proinflammatory index) and DNA methylation.

Methods: A discovery epigenome-wide association study was designed including 645 individuals of the REGICOR population-based cohort. Peripheral blood cell DNA methylation was analyzed using the Illumina HumanMethylation450 BeadChip. Cholesterol efflux and proinflammatory capacities were analyzed in plasma using standardized methods. Robust multivariate linear regression models adjusted for age, sex, smoking, HDL-c levels and surrogate variables were used in the statistical analyses. We declared as statistically significant those associations with p-values that fulfill Bonferroni criteria ( $<1.17E-07$ ).

Results: We discovered two CpGs located in two genes (PEX5 and HOXA3) related to cholesterol efflux capacity; and one CpGs located in GABRR1 related to the proinflammatory index. These CpGs explained 4.94% and 0.83% of the variability of the cholesterol efflux capacity and of the proinflammatory index, respectively.

Conclusions: We have identified three potential loci associated with HDL functionality located in the genes PEX5, HOXA3 and GABRR1. Additional studies are warranted to validate these findings in other populations.

**P17.19****Distinct trends of DNA methylation patterning in the innate and adaptive immune systems**

*R. P. Schuyler<sup>1</sup>, A. Merkell<sup>1</sup>, E. Raineri<sup>1</sup>, L. Altucci<sup>2</sup>, E. Vellenga<sup>3</sup>, J. H. A. Martens<sup>4</sup>, F. Pourfarzad<sup>5</sup>, T. W. Kuijpers<sup>5</sup>, F. Burden<sup>6,7</sup>, S. Farrow<sup>5,7</sup>, K. Downes<sup>6,7</sup>, W. H. Ouwehand<sup>6,7</sup>, M. Frontini<sup>8,7</sup>, H. G. Stunnenberg<sup>4</sup>, J. I. Martín-Subero<sup>9</sup>, I. Gut<sup>1</sup>, S. Heath<sup>1</sup>;*

<sup>1</sup>Centro Nacional de Análisis Genómico, Barcelona, Spain, <sup>2</sup>Seconda Università degli Studi di Napoli, Napoli, Italy, <sup>3</sup>University of Groningen, Groningen, Netherlands,

<sup>4</sup> Radboud University, Nijmegen, Netherlands, <sup>5</sup>University of Amsterdam, Amsterdam, Netherlands, <sup>6</sup>University of Cambridge, Cambridge, United Kingdom, <sup>7</sup>National Health Service (NHS) Blood and Transplant, Cambridge, United Kingdom, <sup>8</sup>University of Barcelona, Barcelona, Spain.

DNA methylation and the localization and post-translational modification of nucleosomes are interdependent epigenetic factors contributing to the generation of distinct phenotypes from genetically identical cells. The BLUEPRINT Epigenome project has produced DNA methylation maps (whole genome bisulfite sequencing) and histone modification maps (chromatin immunoprecipitation sequencing for six post-translational modifications) for nearly 200 samples, covering a range of immune system effector cells and the cancers that arise from them. Here we analyze the global evolution of DNA methylation patterns and their relationship with stably positioned nucleosomes during lineage commitment and maturation of 112 hematopoietic samples. The innate and adaptive lineages of the human immune system show distinct trends, both globally and in relation to consistently positioned nucleosomes, including a progressive loss of methylation in developing lymphocytes. Cancer samples from the two lineages become further polarized, suggesting the involvement of opposing lineage-specific epigenetic mechanisms.

Although DNA methylation is generally restricted to CG dinucleotides, we note the consistent occurrence of non-CG methylation in naïve T lymphocytes and uncommitted hematopoietic progenitor cells, at levels similar to those seen in neurons and stem cells. We also examine sporadic occurrences of localized non-conversion in a few samples, suggesting the possibility of dense exon-specific non-CG methylation. We anticipate broad utility for this resource as a basis for further comparative analyses.

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**P17.21****Association of DNMT1/3a and H3K4 methyltransferase levels with size, grade and phenotype of invasive ductal carcinoma of breast**

*K. Kankava, T. Kvaratskhelia, E. Kvaratskhelia, E. Abzianidze;*

*Tbilisi State Medical University, Department of Molecular and Medical Genetics, Tbilisi, Georgia.*

Introduction: The aim of our pilot study is to analyze correlation of changes in the levels of methyltransferases in nuclear extracts with different morphological characteristics of breast cancer.

Materials and Methods: 21 breast cancer patients and 10 healthy controls were selected for the study. The levels of DNMT1, DNMT3a and H3K4 were measured in nuclear extracts of peripheral blood mononuclear cells (PBMC). Nuclear extracts were prepared using nuclear extraction kit (Abcam). ELISA based DNMTs assay kits (Abcam) were used to measure the amount of DNMT1/3a and H3K4 methyltransferases. Three pathologists performed blind assessment of tumors' morphological and phenotypic characteristics. Results: The results showed that the level of DNMT1 was highest in the control group but didn't correlate with the grade. The level of DNMT1 increased together with the level of Estrogen receptor expression. DNMT3a was found in highest level in Grade III cancer group, followed by Grade II and Grade I groups. In the control group the level of DNMT3a was also rather low. An opposite pattern was seen for H3K4 methyltransferase. DNMT3a level was higher in larger tumors, while the level of H3K4 methyltransferase was lowest. In HER2neu positive cases levels of both - DNMT1 and DNMT3a were increased, while H3K4 methyltransferase was decreased.

Conclusions: This primary study showed that there are some changes in methyltransferase levels in PBMC from breast cancer patients. Continuing observation is planned to specify this and possibly identify an additional diagnostic/prognostic feature for breast cancer.

## P17.22

## Epigenetic Alterations in Human Thyroid Oncogenesis

*A. Botezatu<sup>1</sup>, I. V. Iancu<sup>1</sup>, O. Popa<sup>2</sup>, A. Plesa<sup>1</sup>, D. Manda<sup>2</sup>, M. Bostan<sup>1</sup>, S. Vladoiu<sup>2</sup>, I. Huica<sup>1</sup>, G. Anton<sup>1</sup>, C. Badiu<sup>2</sup>,*

<sup>1</sup>"Stefan S. Nicolau" Institute of Virology, Bucharest, Romania, <sup>2</sup>"Cl Parhon" National Institute of Endocrinology, Bucharest, Romania.

Thyroid carcinoma is the most common endocrine malignancy worldwide with the most common histological type - papillary thyroid cancer (PTC). Aberrant methylation of tumor suppressor genes (TSG) is a hallmark for many types of cancers.

We aim to profile tumor samples and evaluate the methylation status for 22 TSG promoters (APC, BRCA1, CDH1, CDH13, CDKN2A, DAPK, ESR1, FHIT, GSTP1, MGMT, MLH1, NEUROG1, PDLIM4, PTEN, RARB, RASSF1, RUNX3, SOCS1, TIMP3, TP73, VHL, WIF1), to correlate methylation level with biological phenotypes.

We performed a methylation analysis (Human TSG EpiTect Methyl II Signature PCR Array-Qiagen) in PTC samples compared with normal thyroid tissue. Preliminary, we evaluated the promoter methylation for TP73, PDLIM4, WIF1 genes in 120 patient's samples, consisting of 60 PTC and follicular adenoma specimens and their adjacent normal tissue, with qMS-PCR using bisulphite treated DNA samples.

The methylation percentage was found to be increased in PTC samples than control ( $p<0.001$ ). Higher methylation percentage (MP) values were found for TP73 (85.16%) BRCA1 (76.94%), WIF1 (75.8%), PDLIM4 (67.74%).

The methylation level was correlated with tumor grade. TP73 gene promoter methylation seem to be a characteristic for follicular adenoma samples and PTC follicular variant. Methylation of PDLIM4 gene promoter was found from the incipient state of neoplasia, also found in a higher percentage in patients with PTC and PTC follicular variant.

These results illustrate the involvement of epigenetic alterations in thyroid oncogenesis. We consider that the TSG promoter's methylation profile may be a starting point for diagnosis, prognosis of PTC.

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## P17.23

## Differential expression of microRNAs in Familial Mediterranean fever patients

*Y. Akkaya Ulum, E. Batu, O. Karadag, F. Kara, A. Ertenli, S. Kiraz, S. Ozen, E. Yilmaz, B.*

*Balci Peynircioglu;*

*Hacettepe University, Ankara, Turkey.*

Introduction: Familial Mediterranean fever (FMF), one of the most common autoinflammatory disorders, is an autosomal recessively inherited disease. Phenotypic heterogeneity is very important in FMF. Different modifier factors such as epigenetic factors need to be investigated for their role in influencing the phenotype variability seen in FMF patients. Alteration of microRNAs (miRNAs) in biological processes can lead many disorders including inflammatory disorders. We aimed to explore the potential involvement of miRNAs in pathogenesis of FMF.

Materials and Methods: The expression levels of miRNAs in total blood, obtained from healthy controls, patients (homozygotes and heterozygotes) and healthy heterozygotes, were compared with GeneChip miRNA 2.0 Array (Affymetrix). The raw data was analyzed by Multi Experiment Viewer (MeV) and miRNA target genes were determined in miRWALK and clustered in DAVID v6.7 through BioCarta and KEGG pathway maps.

Results: MiR-20a-5p, miR-197-3p, let-7d-3p, miR-574-3p were significantly differentially expressed in homozygous patients and heterozygous patients. Array results were validated using qPCR. All these miRNAs were found to be known regulators in TGF-beta and Toll-like receptor signaling pathway, apoptosis and actin cytoskeleton regulation.

Conclusions: We showed for the first time that there are differentially expressed miRNAs which may target mRNAs clustered in inflammatory pathways such as cytokine secretion, apoptosis and cell migration. Thus our findings provide initial evidence that miRNAs may play role in FMF pathogenesis.

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## P17.24

## The potential role of mir-548c-5p as a regulator of FOXC2 transcription to control podocyte differentiation

*A. Christofides<sup>1</sup>, G. Papagregoriou<sup>1</sup>, H. Dweeb<sup>2</sup>, N. Gretz<sup>2</sup>, K. Felekkis<sup>3</sup>, C. Deltas<sup>1</sup>;*

<sup>1</sup>Molecular Medicine Research Center, University of Cyprus, Nicosia, Cyprus, <sup>2</sup>Medical Research Center, University of Heidelberg, Mannheim, Germany, <sup>3</sup>Department of Life and Health Sciences, University of Nicosia, Nicosia, Cyprus.

Podocytes are highly differentiated epithelial cells outlining the glomerular vessels. FOXC2 is a transcription factor essential for inducing podocyte differentiation. We hypothesize that the transcription of FOXC2 and consequently the differentiation of podocytes, can be controlled by miR-548c-5p through a predicted 21nt long target region located 8kb upstream the FOXC2 transcription start point. By using luciferase reporter constructs, it became apparent that the DNA target site acts as a conventional miRNA-binding site. During early differentiation of AB8/13 human podocytes, miR-548c-5p mimics effectively diminished endogenous FOXC2 levels, while a more dynamic model of this interaction was observed when investigating other time points, suggesting a dependence on target site availability. Therefore, the role of this miRNA target site as a distal enhancer/repressor element was investigated using Chromosome Conformation Capture. Evidence indicates a correlation between podocyte differentiation events and the interaction levels between the miRNA target site and the FOXC2 proximal promoter regions.

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## P17.27

## Identification of novel candidate genes for Hirschsprung disease by differential expression analyses in human enteric precursor cells

*A. Torroglia<sup>1,2</sup>, R. M. Fernández<sup>1,2</sup>, L. Villalba-Benito<sup>1</sup>, M. J. Moya-Jiménez<sup>3</sup>, G. Antón<sup>1,2</sup>, S. Borrego<sup>1,2</sup>;*

<sup>1</sup>Institute of Biomedicine of Seville (IBIS), University Hospital Virgen del Rocío/CSIC/University of Seville, Seville, Spain, <sup>2</sup>Centre for Biomedical Network Research on Rare Diseases (CIBERER), Seville, Spain, <sup>3</sup>University Hospital Virgen del Rocío, Seville, Spain.

Hirschsprung disease (HSCR: OMIM 142623), the most common neurocristopathy in humans (1:5000 newborns), is attributed to a failure of neural crest cells to migrate, proliferate, differentiate or survive in the bowel wall during embryonic Enteric Nervous System (ENS) development. This process requires a wide and complex variety of molecules and signaling pathways which are activated by transcription factors. Although some genes involved in this pathology are well characterized, many aspects remain poorly understood. In this study we aimed to identify novel genes implicated in the pathogenesis of Hirschsprung disease. For this goal, through TaqMan Gene Expression Assays, a differential expression study was performed on a set of genes involved in human stem cells pluripotency as well as on a set of genes encoding transcription factors that participate in different stages of the colonization process. These assays were conducted on Neurosphere-like bodies and gut tissues from both HSCR and control individuals. A total of eleven genes resulted to present statistically significant differential expression among patients and controls. Five of them are implicated in cell proliferation and migration (NESTIN, FN1, LAMC1, PECAM, and SEMA3A), four encode for transcription factors (CDYL, MEIS1, STAT3 and PAX6) and one encodes for a de novo methyltransferase (DNMT3B). This approach allowed us to characterize the precursors of human ENS and to identify a group of excellent candidate genes to be implicated in the ENS development and in the onset of HSCR.

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## P17.28

## A transcription factor binding site-driven approach to identify cis-regulatory variants

*S. Kumar<sup>1,2</sup>, G. Ambrosini<sup>1,2</sup>, P. Bucher<sup>1,2</sup>;*

<sup>1</sup>Swiss Institute for Experimental Cancer Research, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland, <sup>2</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Introduction: Transcription factor (TF)-target site interactions are the elementary molecular events of gene regulation. Identification of SNPs disrupting such interactions and having an effect on chromatin state may shed light on the mechanistic principles of regulatory genetic variation.

Methodology: We developed a discovery pipeline for extracting SNPs that abolish, create, or significantly change the affinity of predicted TF binding sites. Input is a comprehensive variant list, a reference genome plus a set of position weight matrices defining the binding specificity of TFs. Output is a subset of SNPs along with their predicted effects on TF binding. This subset is then used in GWAS studies to identify various kinds of cis-regulatory QTLs. Unlike other approaches, our pipeline can identify de novo created TF binding sites not present in the reference genome.

Results: To test the pipeline we selected 48 TFs and extracted a list of 248,676 SNPs affecting TF binding. As phenotypes we used ChIP-seq data from Waszak et.al. (Cell, 2015) for 45 genotyped individuals. We identified

multiple TF binding variants that are associated with histone modifications: 3799 for H3K27ac, 2187 for H3K4me3 and 476 H3K4me1 (FDR≤0.01). Interestingly, SNPs affecting binding sites of BRCA1 and IKZF1 showed high association rate, suggesting that these TFs mostly act through histone modifications.

Conclusions: Our approach is effective in identifying *cis*-regulatory SNPs that affect predicted TF binding sites. Our SNP list with annotated effects on TF binding may also help in elucidating the mode of action of already known regulatory QTLs. Grant reference: SNSF-CRSII3\_154500.

### P17.29

#### Multilocus methylation imprinting disturbances detected by a reliable and cost effective MassARRAY approach in a study group of 40 patients with imprinting disorders

*L. Fontana<sup>1</sup>, S. Tabano<sup>1</sup>, F. M. Elli<sup>2</sup>, N. Carlessi<sup>3</sup>, L. Paganini<sup>1,3</sup>, R. Falcone<sup>1,3</sup>, C. Pesenti<sup>1,3</sup>, E. Bonaparte<sup>1,3</sup>, S. M. Sirchia<sup>4</sup>, A. Selicorni<sup>5</sup>, S. Maitz<sup>6</sup>, D. Milani<sup>6</sup>, M. Bedeschi<sup>7</sup>, G. Mantovani<sup>2</sup>, M. Miozzo<sup>1,3</sup>;*

<sup>1</sup>Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy, <sup>2</sup>Endocrinology and Diabetology Unit, Department of Clinical Sciences and Community Health, Università degli Studi Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>3</sup>Division of Pathology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>4</sup>Milan, Italy, <sup>5</sup>Medical Genetics, Department of Health Sciences, Università degli Studi di Milano, Milan, Italy, <sup>6</sup>Milan, Italy, <sup>7</sup>Clinical Pediatric Genetics Unit, Pediatric Clinics, MBBM Foundation, San Gerardo Hospital, Monza, Italy, Milan, Italy, <sup>8</sup>Pediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, Milan, Italy, <sup>7</sup>Medical Genetics Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, Milan, Italy.

Introduction. Imprinting disorders (IDs) are syndromes characterized by (epi)genetic alterations affecting imprinted genomic regions. Most of ID patients show epimutations limited to the disease-specific locus, but several exhibit multilocus methylation imprinting disturbances (MLIDs). MLID may account for the variable presentation and the phenotypic overlap between IDs, that make clinical diagnosis often difficult and highlight the need for a reliable method for methylation analysis at multiple imprinted loci.

Materials and Methods. We developed a quantitative methylation test by MassARRAY approach to detect alterations at 12 imprinted regions in 15 pre-and post-natal patients with Beckwith-Wiedemann syndrome (BWS) and 25 patients with pseudohypoparathyroidism type 1 (PHP1), previously diagnosed by pyrosequencing or MLPA. The MassARRAY methylation platform has been set up on 40 normal controls to define methylation ranges at each analyzed CpG site.

Results. 33% of BWS patients showed MLID, with loss of methylation at maternally imprinted loci only. PHP1 patients exhibited both gain and loss of methylation at maternally/paternally imprinted loci and MLID was more frequent in patients with complex clinical presentation.

Conclusions. MassARRAY platform allows a higher frequent detection of MLID in ID patients compared to literature data, thanks to the accurate analysis of several imprinted genes. In BWS patients the detection of MLID confined to maternally imprinted loci, suggests alterations in trans-acting factors involved in the methylation establishment/maintenance in the oocyte. Next generation sequencing is ongoing to identify alterations at these factors. Conversely, MLID patterns in PHP1 patients suggest different/multiple causative mechanisms.

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### P17.30

#### Histone H4 hyperacetylation is altered in patients with impaired spermatogenesis

*F. Barrachina<sup>1</sup>, C. Mallofré<sup>2</sup>, O. Bogle<sup>1</sup>, A. Goudarzi<sup>3</sup>, A. Soler-Ventura<sup>1</sup>, A. Melnick<sup>4</sup>, J. Corral<sup>4</sup>, S. Rousseaux<sup>3</sup>, J. Ballesca<sup>5</sup>, R. Oliva<sup>1</sup>;*

<sup>1</sup>Molecular Biology of Reproduction and Development, IDIBAPS, Faculty of Medicine, University of Barcelona, and Hospital Clinic, Barcelona, Spain, <sup>2</sup>Department of Anatomic Pathology and Otorhinolaryngology, University of Barcelona, and Hospital Clinic, Barcelona, Spain, <sup>3</sup>Institut National de la Santé et de la Recherche Médicale U823 and Université Grenoble Alpes Institut Albert Bonniot, Grenoble F-38700, France,

<sup>4</sup>Department of Urology, IDIBAPS, Hospital Clínic, Barcelona, Spain, <sup>5</sup>Clinic Institute of Gynaecology, Obstetrics and Neonatology, Hospital Clínic, Barcelona, Spain.

Spermatogenesis is a differentiation process characterized by extremely marked chromatin and cellular changes leading to the highly specialized sperm cell. Histone H4 hyperacetylation (acH4) during spermatogenesis is involved in gene activation and in sperm chromatin remodelling in the final stages, and therefore may affect the imprinting of the mature sperm cell. Thus, we hypothesized that acH4 expression pattern during sperma-

togenesis is different between normal and pathological samples. We used a semi-quantitative approach to compare both the intensity and distribution of acH4 immuno-detection in testicular biopsies (n=23) with normal and abnormal spermatogenesis (hypospermatogenesis, spermatogenic arrest and Sertoli cell-only Syndrome). Immunofluorescence was used to further localize acH4 in ejaculated sperm cells. AcH4 immuno-detection was high in spermatogonia, decreased in spermatocytes, and increased again reaching a maximum in elongating spermatids in normal spermatogenesis. The immuno-detection of acH4 in all spermatogenic cell types decreased in patients with hypospermatogenesis or spermatogenic arrest, but increased in the presence of tubular atrophy and fibrosis. Stronger acH4 immuno-detection was observed in Sertoli cells from patients with Sertoli cell-only Syndrome (absence of germinal cells). The localization of acH4 in the mature sperm nucleus indicates the acetylation of retained H4 histones. In conclusion, we found that acH4 is deregulated in patients with altered spermatogenesis. The results from this study implicate acH4 involvement in the remodelling of chromatin during the histone-to-protamine transition through spermatogenesis which may play a role in chromatin imprinting anomalies. Supported by EU-FP7-PEOPLE-2011-ITN-289880, Ministerio de Economía y Competitividad PI13/00699, Fundación Salud 2000 13-015 and EUGIN-UB to RO.

### P17.31

#### A long non-coding RNA that is associated with susceptibility to celiac disease

*A. Castellanos-Rubio<sup>1</sup>, N. Fernandez-Jimenez<sup>2</sup>, R. Kratchmarov<sup>1</sup>, X. Luo<sup>3</sup>, G. Bhagat<sup>1</sup>, P. H. R. Green<sup>1</sup>, R. Schneider<sup>4</sup>, M. Kiledjian<sup>3</sup>, J. Bilbao<sup>2</sup>, S. Ghosh<sup>1</sup>;*

<sup>1</sup>Columbia University, NYC, NY, United States, <sup>2</sup>UPV-EHU, Leioa, Spain, <sup>3</sup>Rutgers University, Piscataway, NJ, United States, <sup>4</sup>NYU, NYC, NY, United States.

Recent studies have begun to implicate long noncoding RNAs (lncRNAs) as regulators of many important biological processes. In this study we have identified a novel lncRNA (lnc13), harboring a celiac disease-associated SNP block, that helps maintain expression of certain inflammatory genes at basal levels. lnc13 regulates gene expression by binding to a member of the family of ubiquitously expressed heterogeneous nuclear ribonucleoproteins (hnRNPs) that interacts with the NuRD chromatin-remodeling complex. Under inflammatory conditions, the level of lnc13 is decreased, thereby removing the repressive effect of lnc13 bound to the hnRNP and enhancing the inflammatory environment. Interestingly, lnc13 levels are significantly decreased in celiac disease intestinal mucosa suggesting that downregulation of lnc13 contributes to the inflammation seen in celiac disease. Furthermore, the lnc13 risk variant binds the hnRNP less efficiently, thus helping to explain the contribution of the SNPs to celiac disease and showing a new mechanism of gene expression control by a SNP located in a non-coding transcript.

### P17.33

#### LncRNAs expression profiles in pancreatic cancer

*I. V. Iancu<sup>1</sup>, A. Botezatu<sup>1</sup>, A. Plesa<sup>1</sup>, C. C. Diaconu<sup>1</sup>, G. Anton<sup>1</sup>, R. Florea<sup>2</sup>, V. Ilie<sup>2</sup>, A. Sorop<sup>2</sup>, V. Tica<sup>2</sup>, S. O. Dima<sup>2</sup>, I. Popescu<sup>2</sup>;*

<sup>1</sup>“Stefan S. Nicolau” Institute of Virology, Bucharest, Romania, <sup>2</sup>Fundeni Clinical Institute, Gastroenterology and Hepatic Transplantation Center, Bucharest, Romania.

Introduction: Pancreatic cancer represents an aggressive disease, being the seventh leading cause of cancer deaths, and its early detection remains a major challenge. Development and progression of pancreatic adenocarcinoma (PDAC) involve gradual accumulation of various genetic and epigenetic alterations. Despite the accumulating evidence that links long non-coding RNAs (lncRNAs) expression to neoplastic transformation, little is known about the role of lncRNAs in PDAC.

The aim of this study is to analyse lncRNAs expression profiles in pancreatic cancer.

Materials and Methods: From 5 paired samples (normal/pancreatic adenocarcinoma) total RNA was obtained and lncRNAs expression levels were assessed using Human LncProfiler qPCR Array Kit (System Biosciences). For lncRNAs validation qRT-PCR was used.

Results: 42 lncRNAs presented significant differences in expression levels between PDAC tissues and corresponding normal tissues (> 2-fold change; p < 0.05). LncRNAs expression profiling showed that 27 were significantly upregulated (among them: Alpha 280, E2F4 antisense, Evf1, Evf2, Hoxa11as, Tsix, Meg3, Malat1) while 15 were significantly downregulated (EgoA, SRA, Lust, PGCEM1, Gomafu, and Tmevpg1). In order to validate the results, two lncRNAs (Malat1 and Meg3) were selected and expression levels were evaluated for 30 sample pairs. Results indicate that both lncRNAs display a high expression level in pancreatic cancer compared with adjacent normal tissues (p < 0.001; respectively p=0.02) especially in advanced stages of the

disease (III-IV).

Conclusions: In this study different lncRNAs expression patterns were associated with PDAC, thus implying a potential biomarker role for these epigenetic factors in pancreatic cancer.

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#### P17.34

#### Correlation of metastasis-related miR-205 with different clinical outcome in primary cutaneous malignant melanoma

B. Sánchez Sendra<sup>1</sup>, C. Monteagudo<sup>2</sup>;

<sup>1</sup>INCLIVA, Valencia, Spain, <sup>2</sup>Pathology Department, Medicine Faculty, University of Valencia, Valencia, Spain.

Introduction: Cutaneous malignant melanoma has a high propensity to spread throughout the body causing a high mortality. There is cumulative evidence that miRNAs, which control many pathological processes acting as gene expression regulators, mediate melanoma invasion and metastasis. Here, we sought to investigate the role of miR-205 in governing patient clinical outcome at the time of primary tumor as a way to differentiate patients with higher risk.

Materials and Methods: We obtained frozen primary human melanoma samples from 65 patients. We selected miR-205 as a good metastasis-related miR. We determined miR-205 expression of these primary tumors by miRNA RT-qPCR and these results were correlated with the clinical outcome.

Results: miR-205 expression was detected in all cases. 49 cases did not metastasize and 16 cases metastasized (24,6%) to distant organs. Clinical follow-up ranged from 10 to 134 months (mean 88 months). From our data, miR-205 was differentially expressed between metastatic versus non-metastatic primary melanomas. It was down-regulated in the former compared with the latter. Furthermore, the cumulated survival stratified by miR-205 expression is significantly different between both primary melanoma groups (Kaplan-Meier curves, Log-rank test).

Conclusions: The miR-205 expression in primary melanoma tumors is able to segregate patients regarding their metastatic ability. Altogether, these findings unravel a key role for miR-205 in distant metastatic melanoma. Given its critical role in metastatic melanoma, miR-205 holds potential as therapeutic target and biomarker.

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#### P17.35

#### Cyclic and constant increased glucose levels variation has important effects DNA methylation profiles

P. Rentero-Garrido<sup>1</sup>, G. DeMarco<sup>1</sup>, L. Olivares<sup>1</sup>, V. Adam<sup>1</sup>, C. Iborra<sup>2</sup>, F. Chaves<sup>1,3</sup>;

<sup>1</sup>Biomedical Research Institute INCLIVA, Valencia, Spain, <sup>2</sup>Sequencing Multiplexing S.L., Valencia, Spain, <sup>3</sup>CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Barcelona, Spain.

Introduction: DNA methylation is an epigenetic mechanism used by cells to regulate their interaction with ambient. The methylation of different regions can be changed as a regulatory response. Carbohydrate levels variation introduce important stress to body cells. Our objective is measuring the influence of cyclical variations of glucose levels in DNA methylation patterns. Material and methods: we cultured HUH7 cells (derived from hepatocytes), and subjected them to different conditions: 5 mM glucose, 30 mM glucose and 30 mM mannitol used as a control, all of them with constant supply or cyclical supply every 8 hours for 7 days. Subsequently, the cell pellet was collected and DNA extracted. To study the patterns of DNA methylation we used bisulfite treatment and Illumina Human Methylation450K BeadChip was performed. Bioconductor packages for debugging, normalization and statistical analysis of the methylation data were used.

Results: Among the different analyses we have found 36 CpG sites delta beta greater than 20% and p.value less than 0.05 and 51 regions with p value less than 0.005, among which genes are related liver carcinogenesis (HEPN1) activating enzymes of protein kinases (PAK3), enzymes pyruvate cycle (PC), angiogenesis inhibitors (ISM1) and enzymes of oxidative stress (NR2C2).

Conclusion: Peaks in energy inputs vary methylation patterns of enzymes and proteins involved in important cellular pathways and the modification produced by cyclical and constant carbohydrate increase are different. These data can have important applications in human health and to understand the mechanisms involved in metabolic diseases.

#### P17.36

#### MicroRNA profiling in patients with upper tract urothelial carcinoma associated to Balkan endemic nephropathy

K. Popovska-Jankovic<sup>1</sup>, P. Noveski<sup>1</sup>, L. Jankovic-Velicovic<sup>2</sup>, S. Stojnev<sup>2</sup>, R. Cukuranovic<sup>3</sup>,

V. Stefanovic<sup>4</sup>, D. Toncheva<sup>5</sup>, R. Staneva<sup>5</sup>, M. Polenakovic<sup>1</sup>, D. Plaseska-Karanfilska<sup>1</sup>;  
<sup>1</sup>Macedonian Academy of Sciences and Arts, Research Centre for Genetic Engineering and Biotechnology, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>Institute of Pathology, Faculty of Medicine, University of Nis, Nis, Serbia, <sup>3</sup>Clinic of Nephrology, Medical Faculty, University of Nis, Nis, Serbia, <sup>4</sup>Department of Clinical Research, Faculty of Medicine, University of Nis, Nis, Serbia, <sup>5</sup>Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria.

Balkan endemic nephropathy (BEN) is a disease that affects people that live in the alluvial plains along the tributaries of the Danube River in the Balkan region. BEN is a chronic tubulointerstitial disease with a slow progression to terminal renal failure and has strong association with upper tract urothelial carcinoma (UTUC). Aberrantly expressed miRNAs have been shown to be associated with many types of cancers including UTUC.

A total of 15 FFPE kidney biopsies were included in this study [4 with normal kidney tissues, 7 with BEN-UTUC and 4 with UTUC from non-endemic Balkan regions (non-BEN-UTUC)]. Total RNA was extracted using commercial FFPE DNA/RNA Kit from Qiagen. Microarray analysis was performed using Agilent SurePrint G3 Human v16 miRNA miRNA 8x60K microarray kit. Statistical analysis of the microRNA expression data was performed using R Bioconductor software and GeneSpring v12.5 software.

Statistical analysis using both softwares revealed 10 microRNAs in BEN-UTUC (hsa-miR-205<sup>↑</sup>, hsa-miR-4322<sup>↑</sup>, hsa-miR-99b<sup>↑</sup>, hsa-miR-3620<sup>↑</sup>, hsa-miR-373<sup>↑</sup>, hsa-miR-3656<sup>↑</sup>, hsa-miR-1290<sup>↑</sup>, hsa-miR-30a<sup>↓</sup>, hsa-miR-127-3p<sup>↓</sup>, hsa-miR-1541<sup>↓</sup>) and 15 in non-BEN-UTUC patients (hsa-miR-205<sup>↑</sup>, hsa-miR-205<sup>↑</sup>, hsa-miR-224<sup>↑</sup>, hsa-miR-224<sup>↑</sup>, hsa-miR-197<sup>↑</sup>, hsa-miR-182<sup>↑</sup>, hsa-miR-183<sup>↑</sup>, hsa-miR-96<sup>↑</sup>, hsa-miR-203<sup>↑</sup>, hsa-miR-149<sup>↑</sup>, hsa-miR-141<sup>↑</sup>, hsa-miR-200c<sup>↑</sup>, hsa-miR-1260<sup>↑</sup>, hsa-miR-210<sup>↑</sup>, hsa-miR-663b<sup>↓</sup>) compared to normal samples, that showed significant difference in the expression ( $p < 0.05$ ) and at least two log-fold changes.

In conclusion, miRNA signature determined in BEN-UTUC patients differ from the non-BEN-UTUC patients; only miR-205 was mutual in both groups. This might impose that different mechanisms/pathways could be responsible for the process of carcinogenesis in UTUC in patients from BEN regions.

#### P17.37

#### Is there any association between nasal polypsis and ADAMTS gene expressions?

A. Tatar<sup>1</sup>, S. Tasdemir<sup>2</sup>, I. Sahin<sup>2</sup>, Z. I. Ocak<sup>3</sup>;

<sup>1</sup>Ataturk University Department of Otorhinolaryngology, Erzurum, Turkey, <sup>2</sup>Ataturk University Department of Medical Genetics, Erzurum, Turkey, <sup>3</sup>Kanuni Sultan Suleyman Research Hospital Department of Medical Genetics, Istanbul, Turkey.

Introduction: Nasal polypsis, which is originated from nose and paranasal sinus mucosa, is a chronic inflammatory disease. It has been accepted a hereditary predisposition because of the increased frequency in some families. A disintegrin and metalloproteinase with thrombospondin type 1 motifs (ADAMTS) genes regulate the structures and functions of different tissues. They are associated with angiogenesis and tumorigenesis, and may therefore be involved in the pathogenesis of nasal polyps. The objective of this study was to show the expression levels of ADAMTS-5, ADAMTS-8 and ADAMTS-9 gene in nasal polypsis and make a contribution to the etiopathogenesis.

Materials and Methods: The study included a total of 48 subjects, of whom 34 patients suffered from nasal polypsis and 14 healthy tissues were control subjects. After histopathological diagnosis, 1x1 cm of nasal polypsis tissue samples used for gene expression studies. The levels of ADAMTS-5, ADAMTS-8 and ADAMTS-9 gene expressions were measured from each sample belonging to the patient and control groups by real time PCR.

Results: We detected an important decrease of expression levels of the ADAMTS-5 and ADAMTS-9 genes in nasal polyp tissues. Expression levels of ADAMTS-8 gene in nasal polyp tissues were similar when compared with the normal nasal tissues.

Conclusion: This is the first time an association has been found between ADAMTS and nasal polypsis. We concluded that ADAMTS-5 and ADAMTS-9 genes may play an important role in the development of nasal polypsis.

## P17.39

**Towards the development of a Lab-on-a-Chip for the identification of trisomy 21 from maternal plasma**

*S. Kyriakou<sup>1</sup>, M. Antonana<sup>2</sup>, M. Ioannides<sup>3</sup>, E. Papageorgiou<sup>1,3</sup>, E. Kyri<sup>1,3</sup>, G. Koumbaris<sup>1,3</sup>, J. M. Ruano-Lopez<sup>2</sup>, P. C. Patsalis<sup>1,3</sup>,*

<sup>1</sup>*The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>IK4-Ikerlan S.COOP, Arrasate Mondragon, Spain, <sup>3</sup>NIPD Genetics LTD, Nicosia, Cyprus.*

**Introduction:** The discovery of circulating fetal DNA in maternal circulation was a breakthrough in the field of prenatal diagnosis. Our group has successfully identified differentially methylated regions (DMRs) between fetal and maternal DNA on chromosomes 13, 18 and 21, and developed a non-invasive prenatal methodology utilizing methylated DNA immunoprecipitation in combination with digital PCR, for the detection of trisomy 21. This study aims to implement MeDIP-dPCR on a Lab-on-a-chip (LOC) for a fast and simplified automated screening.

**Materials and Methods:** The prototype consists of two Chip Units (CU), the MeDIP CU and the pH shift CU. Twenty experiments using  $\lambda$ - meth- and -unmethylated spiked-in samples were used to test the units separately and integrated. Spiked-in samples underwent MeDIP in tube as well as on CUs, followed by qPCR to compare their performance. Finally, CVS spiked-in plasma of non-pregnant women (20%, 10%, 5%), simulating real pregnancies were subjected to MeDIP-dPCR in tube and on the integrated CU system and were evaluated with two 4-plex reactions including six DMRs and two controls.

**Results:** The prototype of the CU revealed an excellent MeDIP performance in terms of control markers and spike-in trend. The comparison of in-tube and on-chip methods exhibited almost identical results as well as excellent reproducibility of the CU protocol.

**Conclusion:** The transfer of MeDIP methodology on a LOC format is promising. Further validation on actual cases will be performed on the CU to assess the performance of the system for the non-invasive prenatal detection of trisomy 21.

## P17.41

**Sapiens-specific genetic changes affecting oxytocin pathways**

*C. Theofanopoulou<sup>1</sup>, C. Boeckx<sup>2</sup>,*

<sup>1</sup>*Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>ICREA/Universitat de Barcelona, Barcelona, Spain.*

**Introduction:** A growing literature suggests that oxytocin pathways (oxt + receptors) must have played a key role in the emergence of human sociality and communication. We wish to provide genetic evidence for this by exploiting recent discoveries in the domain of archaic human genomics.

**Materials and Methods:** We have relied on robust findings in the genetic foundations of language (FOXP2-CNTNAP2 pathway), an extensive database oxytocin-related search (Pubmed), predictive protein interaction program (String 10), and results from archaic human genomics.

**Results:** We put forth a sapiens-specific change (compared to archaic humans) affecting the functional connection between OXT, POU3F2, FOXP2, YY1, and CNTNAP2. POU3F2 is known to be necessary for oxytocin cell differentiation. Crucially, a DNase I hypersensitive site accelerated in the human lineage on Chromosome 6 lies in a gene desert 300 kb from POU3F2, which regulates FOXP2 in a sapiens-unique manner. Two of the substitutions in this site strengthen a putative YY1 transcription factor binding site. YY1 has been implicated in one of the variants of the CNTNAP2 5' promoter as risk factors for autism spectrum disorders. YY1 is among the top 10 enriched transcription factors in differentially methylated regions in sapiens vs. archaic humans. YY1 binds in a methylation-sensitive fashion to an insulator sequence within Peg3. Peg3 knockouts show reduced hypothalamic oxytocin neurons.

**Conclusion:** We suggest that all these oxytocin-related changes are linked and could underlie cognitive and behavioral differences at the heart of our communicative abilities, and open up new avenues in the field of behavioral endocrinology.

## P17.42

**Identification of p53-target genes in *Danio rerio***

*B. Mandriani<sup>1,2</sup>, S. Castellana<sup>3</sup>, C. Rinaldi<sup>1</sup>, M. Manzoni<sup>4</sup>, G. Borsani<sup>4</sup>, E. Monti<sup>4</sup>, T. Mazza<sup>3</sup>, G. Merla<sup>1</sup>, L. Micali<sup>1</sup>,*

<sup>1</sup>*Medical Genetics Unit, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy, <sup>2</sup>PhD Program, Molecular Genetics applied to Medical Sciences, University of Brescia, Brescia, Italy, <sup>3</sup>Bioinformatics Unit I.R.C.C.S. „Casa Sollievo della Sofferenza“, Istituto Mendel, Roma, Italy, <sup>4</sup>Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.*

**Introduction:** To orchestrate the genomic response to cellular stress signals, p53 specifically recognizes and binds DNA containing p53-responsive elements (REs). Differences in RE sequences can strongly impact p53 transac-

tivation capacity and occur even between closely related species. Although p53 functions have been studied in a wide range of species, not much is known about the p53-mediated transcriptional signature in *Danio rerio*.

**Materials and Methods:** We applied an array of computational approaches followed by functional studies to map and characterize p53 binding sites (BS) in the *Danio rerio* genome.

**Results:** A total of 3,033 hits were found in the zebrafish genome. First, 10 predicted p53REs were selected according to defined series conditions and functionally validated by performing luciferase-based assays. All tested sequences were significantly responsive to p53. Next, expression level of candidate genes associated to the predicted p53RE in ZF embryos treated with both R-roscoxitine or injected with p53 antisense morpholino (p53MO), were qPCR profiled, detecting a symmetrical change of mRNA levels in 7 out of 10, respectively.

Based on the first 10 experimentally validated *Danio rerio* p53RE, 21 additional putative p53BS were then selected through a position-specific scoring matrix, and subsequently validated by qPCR. We observed a significant activation in 12 out of 21 candidate genes in embryos treated with R-roscoxitine and a reduced mRNA level of all 21 genes in embryos injected with p53MO. **Conclusions:** This study identified and validated functional p53 responsive elements and novel p53 target genes in the *Danio rerio* genome.

## P17.43

**Transcriptional regulation of GBA and GBAP1 genes**

*V. Rimoldi<sup>1</sup>, G. Rovaris<sup>1</sup>, L. Straniero<sup>1</sup>, S. Goldwurm<sup>2</sup>, G. Soldà<sup>1</sup>, R. Asselta<sup>1</sup>, S. Duga<sup>1</sup>,*

<sup>1</sup>*Department of Biomedical Sciences, Humanitas University, Rozzano, Milan, Italy,*

<sup>2</sup>*Parkinson Institute, Milan, Italy.*

**Introduction:** Parkinson disease (PD) is a complex disorder characterized by the loss of dopaminergic neurons of the substantia nigra. To date, mutations in the GBA gene, encoding the lysosomal glucocerebrosidase, are considered the main genetic risk factor for PD, and glucocerebrosidase downregulation was demonstrated to have a crucial role in PD.

We previously described a miRNA-mediated regulatory circuit linking GBA and its pseudogene (GBAP1) expression. GBA/GBAP1 resulted co-expressed in most tissues (ratio: 2 to 200). Furthermore, we demonstrated that GBAP1 levels are downregulated by the nonsense-mediated mRNA decay (NMD) pathway.

GBA and GBAP1 share a similar genomic structure, characterized by a proximal (P1) and a distal (P2) promoter; no data on the expression regulation of GBAP1 as well as on the relative contribution of the two promoters are available so far.

**Methods:** We cloned 1kb of the 4 promoters in a luciferase vector and produced serial 5'-deletion constructs for each promoter. These plasmids were transfected in different cell lines.

**Results:** At difference with what observed for endogenous transcripts, we demonstrated that P2 promoters are stronger than P1s for both genes, and that GBA and GBAP1 promoters have comparable activities. Two Transcription-Factor EB (TFEB) binding sites resulted the main drivers of P1s transcriptional activity, with TFEB overexpression having a stronger up-regulating effect on GBA transcripts.

**Conclusions:** Besides differences in epigenetic regulation, not faithfully modeled in our system, NMD is likely to be the major determinant of the relative expression of GBA/GBAP1 transcripts.

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## P17.44

**Genome-wide analysis of DNA methylation in prostate cancer using technology infinium humanmethylation450 beadchips**

*R. I. Sultanov<sup>1</sup>, K. A. Babalyan<sup>1</sup>, E. V. Generozov<sup>2</sup>, N. B. Zaharzhevskaya<sup>2</sup>, I. Sharova<sup>2</sup>,*

*M. N. Peshkov<sup>2</sup>, A. O. Vasilev<sup>3</sup>, A. V. Gorovrov<sup>3</sup>, D. Y. Pushkar<sup>3</sup>, E. A. Prilepskaya<sup>2</sup>, D. A.*

*Danilenko<sup>2</sup>, E. A. Babikova<sup>1</sup>, A. K. Larin<sup>2</sup>, V. M. Govorun<sup>2</sup>,*

<sup>1</sup>*The Moscow Institute of Physics and Technology, Dolgoprudny, Russian Federation,*

<sup>2</sup>*Research Institute of Physico-Chemical Medicine Federal Medical-Biological Agency, Moscow, Russian Federation, <sup>3</sup>Department of Urology Medical University „Moscow State Medical and Dental University by A.I. Yevdokimov“ The Ministry of Public Health of Russia, Moscow, Russian Federation.*

Prostate cancer (PCa) is the most common cancer in men worldwide. In Russia, the annual incidence is increasing by an average of 8.7%. A large number of experimental data reveal the role of genetic (mutations) and epigenetic (DNA methylation) factors in the pathogenesis of prostate cancer, which allows us to consider some of them as potential markers for diagnosis.

The study included paired PCa and adjacent benign tissue samples from 12 radical prostatectomy patients. Epigenetic profiling was done using the Infinium HumanMethylation450 BeadChip. Linear models that accounted for the paired study design and False Discovery Rate q-values were used to eval-

luate differential CpG methylation.

In total, 21610 differentially methylated CpG sites were identified in PCA versus adjacent benign tissue ( $q$ -value  $< 0.01$ ), the majority of which were hypermethylated (~85%). 186 top-ranked hypermethylated CpGs had a mean methylation difference of at least 40% between tissue types, and for 14 genes over 50% of promoter region CpGs were hypermethylated. We selected a panel of 9 highly hypermethylated sites. This set was successfully cross-validated using methylation data from 40 paired samples from the prostate cancer project The Cancer Genome Atlas consortium (specificity ~95%, sensitivity ~97%, the area under the ROC curve ~0.96).

In summary, this study reports a large number of loci with novel differential methylation statuses, which may potentially be used for the development of future epigenetic-based diagnostic tests.

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#### P17.46

##### Investigating the role of long non-coding RNA that are genetically associated with rheumatoid arthritis susceptibility

**J. Ding, A. McGovern, P. Martin, S. Eyre, J. Worthington;**  
University of Manchester, Manchester, United Kingdom.

**Introduction and Methods:** Rheumatoid arthritis is a chronic, autoimmune, inflammatory disease that affects ~1% of adults in the UK. Genetic factors account for ~60% of the variation in disease susceptibility and are well defined through a strong history of genetic studies. As is common for complex genetic disorders, the majority of the ~100 loci associated with rheumatoid arthritis susceptibility lie outside of protein coding regions. How these loci contribute to susceptibility is largely unknown. Bioinformatic analysis of susceptibility loci was used to identify and prioritise loci that overlap long non-coding RNA: a class of molecules that have been demonstrated to occupy various important functions. Loci situated some distance from the nearest protein coding genes, and where a physical, DNA looping interaction was observed from in-house generated Capture Hi-C data were prioritised. Nine potentially interesting candidates were subjected to expression analysis, performed using the Nanostring platform, aiming to identify expression in relevant stimulated and unstimulated tissues and cell types, in both total and nuclear RNA.

**Results and Conclusion:** As is typical for long non-coding RNA, expression was generally very low and highly tissue specific. This analysis identifies a selection of long non-coding RNA that will be the focus of future work to investigate the impact of disease risk genetic variance on both the long non-coding RNAs and downstream targets. Of particular interest are lnc-IRF8-5 and lnc-RPLP1-2, which are expressed in synovial tissue, and peripheral blood mononuclear cells, situated over 300kb from their target gene and containing variants significantly associated with RA.

#### P17.47

##### Epigenetic study in patients with sepsis

**D. Tzoneva<sup>1</sup>, D. Nikolova<sup>2</sup>, S. Yankova<sup>2</sup>, Z. Hammudeh<sup>2</sup>, S. Maslyankov<sup>1</sup>, I. Dimova<sup>2</sup>;**  
<sup>1</sup>University Hospital Aleksandrovska, Sofia, Bulgaria, <sup>2</sup>Department of Medical genetics, Medical University Sofia, Sofia, Bulgaria.

Lack of potential diagnostic or prognostic markers for sepsis makes it dreadful disease, which holds the highest mortality. Genetic studies have shown that the type of infecting organism, outcome of infections and mortality can be predetermined by analyzing an individual's genome. Genetic/epigenetic studies build up hope for newer diagnostic/prognostic methods in the treatment of sepsis, as well as understanding of its pathogenesis. In our study we have analyzed DNA methylation changes in 22 genes, connected to cellular stress and toxicity. We collected blood samples from 21 surgical patients with sepsis ending in exitus lethalis and 20 control healthy individuals. The promoter methylation status of 22 genes was analyzed using The Human Stress & Toxicity Pathway Finder

EpiTect Methyl II Signature PCR Array. Seven genes were successfully analyzed in all samples - CSTB, DNAJC15, Gadd45a, Gadd45G, Prdx2, Tp53 and Xpc. The average methylation level for these genes in control subjects was respectively 0, 0.09, 0, 0, 0.07, 0 and 0. In patients with sepsis it was as follows - 0.025, 0.18, 0.1, 0.22, 0.11, 0.1 and 0.06. The highest increase in the methylation fraction was observed for the gene Gadd45G, which encodes cytokine-responsive protein. This study highlights the link between epigenetic changes in inflammatory genes and pathogenesis of sepsis. Further studies are needed for validation of the role for epigenetic markers in the pathogenesis of sepsis. Sepsis genomics will help us in directing treatment against this complication.

#### P17.48

##### Tissue-specific and developmental stage-specific expression of sphingomyelin synthase genes

**I. B. Filippenkova, T. A. Kolomin, E. O. Kalinichenko, V. G. Dmitrieva, L. V. Dergunova, S. A. Limborska;**  
Department of Molecular Bases of Human Genetics, Institute of Molecular Genetics, Russian Academy of Sciences, 123182, Moscow, Russian Federation.

**Introduction:** Sphingomyelin synthase genes (SGMS1 and SGMS2) encode the vital enzymes SMS1 and SMS2 which catalyze the synthesis of sphingomyelin and diacylglycerol from phosphatidylcholine and ceramide. SMS1 and SMS2 are involved in the processes of membrane transport, cell proliferation and apoptosis. However, the structural and functional features of sphingomyelin synthase genes are poorly understood.

**Materials and Methods:** We present a study of mRNA expression of sphingomyelin synthase genes in adult and embryonic tissues using real-time PCR. The transcript amounts were estimated relative to the average mRNA level of housekeeping genes (LDHA, GAPDH and RPL3).

**Results:** Human, rat and mouse adult tissues as well as rat brains at embryonic days 7, 9, 13, 17 and 21 were investigated. In adults we found similar mRNA expression of sphingomyelin synthase genes in non-brain tissues, but the level of the SGMS2 mRNA was significantly lower in the brain tissues. In embryonic rat brains the mRNA expression of sphingomyelin synthase genes is varied in developmental stage-specific manner. We have previously determined the circular RNAs (circRNAs) emerging from exons of the 5' untranslated region of SGMS1 gene. These circRNAs are evolutionarily conserved and highly represented in human, rat and mouse adult brain. The expression of circRNAs for SGMS1 was raised during rat embryonic brain development.

**Conclusions:** Sphingomyelin synthase genes have tissue-specific and developmental stage-specific expression. We assume that the circRNAs are involved in the regulation of sphingomyelin synthase activity in different tissues and developmental stages.

#### P17.49

##### Glycemic control and telomere length in juvenile patients with type 1 diabetes

**T. Tesovnik<sup>1</sup>, J. Kovač<sup>1</sup>, T. Hovnik<sup>1</sup>, K. Dovč<sup>1</sup>, N. Bratina<sup>1</sup>, T. Battelino<sup>1,2</sup>, K. Trebušak Podkrajšek<sup>1,2</sup>;**  
<sup>1</sup>University medical centre Ljubljana, University childrens hospital, Ljubljana, Slovenia,

<sup>2</sup>University of Ljubljana, Faculty of medicine, Ljubljana, Slovenia.

**Introduction:** Type 1 diabetes (T1D) is a chronic disease with characteristic state of hyperglycemia, caused by autoimmune destruction of pancreatic  $\beta$ -cells producing insulin. The chronic state of hyperglycemia has been associated with increased oxidative stress and inflammation and leads to development of diabetic complications. The aim was to evaluate telomere length (TL) and its dynamics as a biomarker in juvenile patients with T1D in relation with glycemic control.

**Materials and Methods:** In our research, we included 44 juvenile T1D patients, 17 with poor ( $\text{HbA1c} > 9\%$ ) and 27 matched patients with good glycemic control ( $\text{HbA1c} < 7.5\%$ ). We assessed 237 chronological DNA samples (3 to 5 per each patient) for chronological analysis of relative TL, telomere dynamics and its comparison with the matched controls.

**Results:** Results indicate no statistical difference of telomere dynamics between matched patients with good and poor glycemic control, but the linear regression of all relative TL measurements indicates the impact of T1D duration, even adjusted to the age of participants (slope = -0.2638,  $r^2 = 0.077$ ,  $p < 0.0001$ ).

**Conclusion:** Results of our study indicate that telomere dynamics is a complex process. We could not confirm a glycemic control related decrease of TL in T1D patients probably due to the short investigated period of time. Nevertheless, the observed relative TL indicates the negative impact of the disease duration on the telomere dynamics, although a larger number of chronological samples over longer period of disease duration is required to confirm our findings.

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## P17.50

## Abnormal methylation status of Rap1Gap gene and its association with thyroid cancer

A. Ghadiri<sup>1</sup>, B. Faam<sup>2</sup>, M. Ghaffari<sup>3</sup>, M. Totonchi<sup>4</sup>, M. Hashemi tabar<sup>5</sup>;

<sup>1</sup>Department of Immunology, Faculty of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran, Ahvaz, Iran, Islamic Republic of, <sup>2</sup>1. Cellular and Molecular Research Center, Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of, <sup>3</sup>3. Department of Clinical Biochemistry, Faculty of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran, Ahvaz, Iran, Islamic Republic of, <sup>4</sup>4. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran; Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Repro, Tehran, Iran, Islamic Republic of, <sup>5</sup>5. Cellular and Molecular Research Center, Faculty of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran, Ahvaz, Iran, Islamic Republic of.

Introduction: Global hypermethylation of DNA has been found in several malignancies [1]. Studies on thyroid tumors have shown controversial results. In this study, we aimed to examine the role of DNA methylation of CpG74a in Rap1gap gene among thyroid cancer subtypes.

Methods: we analyzed 95 thyroid tumor samples including normal thyroid (33 cases), benign nodule (36cases), papillary thyroid cancer (PTC) (20 cases), follicular thyroid cancer (FTC) (3 cases) and Anaplastic thyroid cancer (ATC) (3 cases) from Erfan grand hospital, Tehran, Iran. Rap1gap gene expression was assessed using SYBR Green Real-Time PCR. CpG74a Island within the promoter region of this gene was selected; DNA methylation pattern was examined using methylation specific PCR (MSP).

Results: this study showed that Rap1gap was frequently lost or downregulated in various types of tumors, particularly in the most invasive and aggressive forms of thyroid cancer. DNA methylation status of this CpG showed that this alteration is mostly associated with Rap1gap gene downregulation in the differentiated thyroid cancers (PTC, FTC)(80%). However, this value was 16% in undifferentiated thyroid cancer (ATC). CpG74a was methylated in 66% and 72% of individuals with normal thyroid and benign nodule respectively.

Conclusion: this study demonstrates that Rap1gap is likely to serve as an important tumor suppresser gene in thyroid cells and its downregulation during aberrant methylation contributes to differentiated tumor progression and invasion.

Key words: thyroid cancer; RAP1gap; DNA methylation

1. Keelawat S, et al. Detection of global hypermethylation in well-differentiated thyroid neoplasms by immunohistochemical analysis. *J Endocrinol Invest* 2015; 38: 725-32

## P17.51

## Characterization of telomeric abnormalities in ICF syndrome types II - IV

S. Toubiana, H. Katzir, S. Yehezkel, S. Selig;

Molecular Medicine Laboratory, Rambam Health Care Campus and Rappaport Faculty of Medicine and Research Institute, Technion - Israel Institute of Technology, Haifa, Israel.

ICF (Immunodeficiency, Centromeric instability, Facial anomalies) syndrome type I patients carry biallelic mutations in *DNMT3B* which encodes the enzyme that *de novo* methylates repetitive sequences during embryonic development. ICF type I cells display severely hypomethylated subtelomeric regions, abnormally high TERRA levels and short telomeres and ICF type I fibroblasts enter senescence prematurely. ICF syndrome is genetically heterogeneous and three additional genes responsible for this syndrome have recently been identified; *ZBTB24* (type II), *CDCA7* (type III) and *HELLS* (type IV). These gene products were not previously linked with the processes of DNA methylation. While many phenotypic aspects of ICF types II-IV are similar to type I, the telomeric phenotype of these patients' cells has not yet been explored. To this end we are studying subtelomeric methylation, TERRA expression and telomere length in lymphoblastoid cells, fibroblasts and blood. Our initial findings in lymphoblastoid cells suggest that ICF types II-IV display subtelomeric methylation patterns that vary from those of ICF type I. NBL-1 subtelomeric repeats are hypomethylated as in type I, while the Htel subtelomeric repeats, which contain many of the TERRA promoters, are not. In concordance with the normal methylation of Htel repeats, these cells display normal low TERRA levels. Further characterization of the telomeric phenotype in all cell types will reveal the extent of the telomeric abnormalities in these ICF patients. The findings stemming from this research will further elucidate the mechanisms by which subtelomeric methylation is generated and maintained, and how disrupted subtelomeric methylation can lead to an abnormal telomeric phenotype.

## P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

## P18.001

## Single nucleotide polymorphisms of the WNT5A gene predispose to the acute respiratory distress syndrome in patients with severe sepsis

M. Acosta-Herrera<sup>1,2,3</sup>, B. Guillen-Guio<sup>2</sup>, M. Pino-Yanes<sup>1,2</sup>, V. Sagredo<sup>4</sup>, C. Martin-Rodriguez<sup>5</sup>, L. Nogales<sup>6</sup>, J. Blanco<sup>1,7</sup>, A. Corrales<sup>1,2</sup>, L. Tamayo<sup>7</sup>, J. Collado<sup>8</sup>, E. Gonzalez<sup>9</sup>, E. Espinosa<sup>10</sup>, C. Subira<sup>11</sup>, L. Perez-Mendez<sup>12</sup>, S. Ma<sup>12</sup>, J. Villar<sup>1,3</sup>, C. Flores<sup>1,2</sup>;

<sup>1</sup>CIBERES, Instituto de Salud Carlos III, Madrid, Spain, <sup>2</sup>Hospital Universitario N.S. de Candelaria, S/C de Tenerife, Spain, <sup>3</sup>Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain, <sup>4</sup>Intensive Care Unit, Hospital Clínico Universitario de Salamanca, Salamanca, Spain, <sup>5</sup>Intensive Care Unit, Hospital General Universitario de Ciudad Real, Ciudad Real, Spain, <sup>6</sup>Intensive Care Unit, Hospital Clínico de Valladolid, Valladolid, Spain, <sup>7</sup>Intensive Care Unit, Hospital Universitario Río Hortega, Valladolid, Spain, <sup>8</sup>Intensive Care Unit, Hospital Rio Carrión, Palencia, Spain, <sup>9</sup>Intensive Care Unit, Hospital Virgen de La Luz, Cuenca, Spain, <sup>10</sup>Department Anesthesia, Hospital Universitario NS de Candelaria, S/C de Tenerife, Spain, <sup>11</sup>Intensive Care Unit, Fundació ALTHAIA, Manresa, Spain, <sup>12</sup>Department of Pulmonary/Critical Care, University of Chicago, Chicago, IL, United States.

Introduction. The role of the wingless integration signaling site family, member 5A (WNT5A) protein is central to mechanisms of lung healing and fibrosis, including those triggered during lung damage in the acute respiratory distress syndrome (ARDS). We investigated whether common single nucleotide polymorphisms (SNPs) across the WNT5A gene contribute to the development of ARDS in patients with severe sepsis.

Methods. We conducted a prospective, multicenter genetic association study in a cohort of 387 critically ill patients fulfilling international criteria for severe sepsis. All patients were followed for the development of ARDS. Patients developing moderate and severe ARDS were analyzed as a single group (n=215) and the remaining patients served as controls (n=172). Seven tagging SNPs of the WNT5A gene were genotyped. After imputation, association testing with ARDS susceptibility was examined for 37 SNPs using logistic regression analysis.

Results. We identified three SNPs in high linkage disequilibrium (0.75<r<sup>2</sup><1) that were associated with ARDS susceptibility as a single association signal. The most significant SNP (rs77344209) had an odds ratio of 0.56 for the T allele (95% confidence interval, 0.39-0.80; p=0.001). Based on its location, functionality assessments revealed that this SNP may participate in the modulation of WNT5A gene expression.

Conclusions. Common variants of the WNT5A gene are significantly associated with the development of sepsis-induced ARDS. Further studies in independent population of patients with ARDS are ongoing to confirm our findings.

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## P18.002

## Genetic signals of adaptation to climate in native populations of North Eurasia

V. Stepanov<sup>1,2</sup>, V. Kharkov<sup>1,2</sup>, K. Vagaitseva<sup>1,2</sup>, A. Cherednichenko<sup>1</sup>, A. Bocharova<sup>1</sup>;

<sup>1</sup>Institute for Medical Genetics, Tomsk, Russian Federation, <sup>2</sup>Tomsk State University, Tomsk, Russian Federation.

Introduction: Natural selection probably has played the substantial role in shaping the genetic and phenotypic structure of human populations. The current work aims at search of natural selection signals, providing the adaptation of native North Eurasian populations to cold climate of the region, using candidate genes approach.

Materials and Methods: 28 SNP in genes and genomic regions demonstrating positive signals of natural selection in recent genome-wide studies and involved in pathways and processes potentially implicated in adaptation to cold or resistance to low temperatures (thermoregulation, response to temperature stress, energy metabolism, regulation of muscle constriction etc.) were selected for the study. SNPs were genotyped by multiplex PCR and MALDI-TOF mass spectrometry in 11 native populations representing Northern part of Eurasia - Chukchi, Siberian Eskimo, Nivkh, Koryak, Yakut, Buryat, Khant, Ket, Udmurt, Kirghiz and Uzbek.

Results: The trend of reducing the genetic diversity from southwest to northeast was found. The high total level of genetic differentiation was observed (*Fst* = 0.764). Detection of loci under selection from F-statistics using coalescent simulations to generate the null distribution of F-statistics under the hierarchical island model of population structure reveals signals of

selection in 10 out of 28 genetic markers including MKL1, COL19A1, CPT1A, UCP3, SLC2A12, and MYOF genes, as well as in several RNA coding loci. Conclusions: Genetic diversity genetic regions associated with adaptation to cold climate in North Eurasia populations demonstrate non-neutral patterns in North Eurasian populations.

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#### P18.003

#### Genome-wide identification of microRNA-related variants influencing the risk of age-related macular degeneration

**M. Ghanbari, A. Colijn, O. Franco, A. Dehghan, S. Erkland, C. Klaver, M. M. Meester;**  
Erasmus medical Center, Rotterdam, Netherlands.

Introduction: Age-related Macular Degeneration (AMD) is one of the major causes of blindness worldwide which part of the pathogenesis remains to be elucidated. Polymorphisms in miRNAs and miRNA-binding sites may affect miRNA function and could therefore contribute to disease risk.

Methods: We investigated the extent to which variants located in miRNAs and their binding sites are associated with AMD using the largest available genome-wide association study (GWAS). We integrated our findings with different biological and computational data to provide evidence for the functionality of the identified variants. We further experimentally examined the effect of associated-variants on the expression levels of related miRNAs and target genes.

Results: Out of 2347 miRNA-variants, 431 were present in the AMD-GWAS, we found three variants that were significantly associated with AMD. In-silico analysis indicated that the mutant alleles could impact on the miRNA hairpin structures. Our subsequent analysis highlighted target genes that may mediate the effect of these miRNAs on AMD. We are currently performing functional experiments to validate the effect of variants on the levels of mature miRNAs and the highlighted target genes. In addition, we examined the association of 82,051 miRNA-binding site variants with AMD and identified 76 associated-variants that could potentially affect miRNA-mediated regulation of their host genes. We, based on GWAS results, eQTL analysis and gene expression data, selected two of these variants to experimentally show an allele-specific regulation of the host genes by related miRNAs.

Conclusions: We provide evidence supporting the role of miRNAs in the pathogenesis of AMD.

#### P18.005

#### Estimation of allele frequency in Chinese population and association study using large scale of low-coverage sequencing data of plasma cell-free DNA

**F. Chen<sup>1,2</sup>, H. Xu<sup>1</sup>, Y. Gao<sup>1</sup>, X. Jin<sup>1</sup>, Y. Zhang<sup>1</sup>, P. Xiao<sup>1</sup>, S. Li<sup>1</sup>, L. Fang<sup>1</sup>, X. Xu<sup>1</sup>;**  
<sup>1</sup>BGI-Shenzhen, Shenzhen, China, <sup>2</sup>Section of Molecular Disease Biology, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Introduction: Previous allele frequency of Chinese Population was calculated using whole exome/genome sequencing data from 1000 Genomes Project. Since 2012 to 2013, we have accumulated low-coverage whole-genome sequencing data of more than 140,000 clinical samples. The purpose of this study was to develop a method to calculate allele frequency in Chinese population using this big and low-coverage data.

Method and Material: Plasma cell-free DNA of each sample was sequenced to obtain ~0.1X whole genome sequencing depth. Maximum-likelihood and site frequency spectrum were used to estimate allele frequency, and the results were compared to the 1000 Genome Project results. Genetic hearing loss was used as a disease model to evaluate the accuracy of allele frequency estimation. As the application of this method, allele frequency of height and diabetes was used for association studies.

Result: The allele frequency spectrum of 140,000 samples was consistent to the 1,000 Genomes Project results, while more variants of ultra-low frequency could be detected. Validated by mass spectrometry results, allele frequency ranging from 0.1% to 1% of 11 loci of genetic hearing loss could be accurately detected. Association study of height-related loci revealed five potential targets (ACAN, GABRA1-GABRG2, PMCH-IGF1, FERMT1-BMP2 and FADS1. One suggestive susceptibility locus of diabetes, LAMC2 at rs942617, was also identified.

Conclusion: It is possible to use the big data of low-coverage sequencing results to calculate allele frequency. Our findings were compatible with previous reports in Chinese population and reveal certain features of molecular prevalence of genetic diseases in China.

#### P18.007

#### Discovery of a minimal SNP set for human ancestry assignation using Monte-Carlo Tree Search

**C. Flores<sup>1,2</sup>, S. Basaldúa<sup>1</sup>, M. Moreno-Vega<sup>4</sup>;**

<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain,

<sup>2</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain,

<sup>3</sup>Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, Santa Cruz de Tenerife, Spain, <sup>4</sup>Department of Computer and Systems Engineering, Universidad de La Laguna, Santa Cruz de Tenerife, Spain.

Introduction: Ancestry assignment in medical and forensic applications results inadequate if based on self-perceptions. Using genetic information for the inference usually requires a large number of SNPs, being impractical and cost-prohibitive in some settings. In addition, the most cost-effective SNP sets consistent on less than a dozen SNPs, only permit the assignation to four out of the seven continental groups. Here we aimed to provide a reduced informative SNP set, preserving a sufficient assignment capacity to seven continental groups, providing a technology-unconstrained low-cost solution.

Materials and Methods: We explored the use of a Monte-Carlo Tree Search (MCTS) algorithm, which allowed evaluating millions of combinatorial models based on a tree search automatically bypassing the ancestry information redundancy provided by the SNPs. We utilized 60% of the human DNA samples from the CEPH-HGDP, determined for 650K SNPs, to derive the SNP sets consistent in 5 to 12 SNP combinations. The reminder 40% of the CEPH-HGDP samples was used for testing their assignment capacity based on differences in likelihood assignations to each group. An additional validation was performed in data from the 1000 Genomes Project (1KGP) populations.

Results: The SNP sets with <12 SNPs were able to assign up to 52.3% of the testing sample. We derived six equivalently optimal 12-SNP sets demonstrating, on average, a correct assignment of 87% of CEPH-HGDP and 88% of the 1KGP samples.

Conclusions: The 12-SNP sets constitute novel solutions for blind, cost-effective and precise assignment of a subject's ancestry to one of the seven continental groups.

#### P18.008

#### Determination of mitochondrial haplogroups of individuals from a medieval cemetery on Devín Castle using analysis of ancient DNA

**L. Šebest<sup>1</sup>, M. Baldoví<sup>2</sup>, C. Bognár<sup>1</sup>, L. Kádasj<sup>1,2</sup>, K. Kyselicová<sup>3</sup>, R. Beňuš<sup>3</sup>;**

<sup>1</sup>Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, <sup>2</sup>Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>3</sup>Department of Anthropology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

Introduction: Despite the low concentrations of ancient DNA (aDNA) in historical samples and the risk of its contamination with modern DNA, aDNA provides valuable information from various historical periods. The aim of this study was to isolate and analyse aDNA from human remains from medieval cemetery on Devín Castle (Slovakia) dated to 11th and 12th century for the purpose of mitochondrial haplogroups determination. Due to the proximity of the Devín Castle to the medieval overland a river trade routes, we expected the occurrence of haplogroups atypical for this region.

Materials and Methods: As samples for the aDNA isolation were used 40 human teeth and bones. All worksteps were carried out in accordance with strict recommendations for working with aDNA. Determination of mitochondrial haplogroups was performed on the basis of the HVRI sequencing followed by analysis of polymorphisms in this region.

Results: We determined 11 different haplogroups (H, H1, H1a, H5, J2b, K, T1a, T2, U5a, V and W) with 16 various haplotypes.

Conclusions: Although we detected haplogroups with higher frequencies in regions of present day Hungary (W) and northeastern Germany (H1a), all of the observed haplogroups are commonly distributed in central Europe (where the Devín Castle is also located) and one of the possible explanations of the absence of more „exotic“ haplogroups may be lower importance of Devín Castle as a part of the medieval trade routes.

#### P18.009

#### Ancient DNA study of a mysterious lake of Himalaya.

**N. Rai<sup>1</sup>, G. Chaubey<sup>2</sup>, L. Singh<sup>1</sup>, K. Thangaraj<sup>1</sup>;**

<sup>1</sup>Centre for Cellular and Molecular Biology, Hyderabad, India, <sup>2</sup>Estonian Biocentre, Tartu, Estonia.

The high-altitude (5029 meters) Roopkund lake is situated in the Himalayan Mountains within the Northern Indian state of Uttarakhand. Here, 70 years ago, several hundred human skeletons were found, in the lake itself and in its vicinity. This discovery was puzzling as the reasons for which so many

people would have travelled and found their end there remained elusive. This is the first study to address the origin of these individuals through genetic and biological analysis of the 82 skeletal remains. Using amelogenin marker, it was found that the majority of the individuals were males. In the same time, AMS dating of the bones revealed that the individuals had lived in the 8th century AD. To address the ancestry of this population, DNA was extracted from the bones of 80 individuals and the complete mitochondrial genome of each of them was sequenced; furthermore, for 25 of them, 200,000 autosomal markers were also genotyped. The comparative genetic analysis, which includes modern day data from both 700 individuals living in the vicinity of the Roopkund site and 22,000 individuals from across India, suggests that the 8th century Roopkund population comprised two groups of genetically distinct individuals. The majority showed genetic affinity with present day contemporary higher caste group and also middle eastern populations, while the others displayed common haplogroups with the Austro-Asiatic population of the North Indian Himalayas.

#### P18.011

##### Application of Artificial Neural Networks for revealing risk factors linked to thymic pathology in Myasthenia Gravis

**A. Lopomo**<sup>1,2</sup>, R. Ricciardi<sup>3,4</sup>, M. Maestri<sup>5</sup>, A. De Rosa<sup>3</sup>, E. Grossi<sup>6</sup>, F. Coppede<sup>1</sup>, L. Migliore<sup>1</sup>, <sup>1</sup>Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy, <sup>2</sup>Doctorate school in Genetics, Oncology and Clinical Medicine, Department of Medical Biotechnologies, University of Siena, Siena, Italy, <sup>3</sup>Department of Clinical and Experimental Medicine, Neurology Unit, University of Pisa, Pisa, Italy, <sup>4</sup>Division of Thoracic Surgery, Robotic Multidisciplinary Centre for Surgery, Cardiotoracic and Vascular Surgery Department, University of Pisa, Pisa, Italy, <sup>5</sup>Semeion Research Centre of Sciences of Communication, Rome, Italy.

**Introduction:** Myasthenia Gravis (MG) is a rare autoimmune disease characterized by muscle weakness and fatigue, is B-cell mediated, and is associated with autoantibodies to components of the postsynaptic muscle endplate (in most cases, directed to the acetylcholine receptor, AChR). The role of thymus in the pathology is highlighted by the presence of frequent histological abnormalities, as thymoma and hyperplasia, and by the benefit of thymectomy. To reveal risk factors linked to thymic pathology in MG, we applied a mathematical approach based on an artificial adaptive system called Auto Contractive Map-Auto-CM algorithm (Auto-CM), a special kind of Artificial Neural Network (ANN) able to find associations among variables.

**Materials and methods:** A total of 491 AChR<sup>+</sup> MG patients were characterized for age, gender, onset of pathology, Osserman classification of MG and thymic pathology. They were also genotyped for *MTHFR* 677C>T, *MTRR* 66A>G, *MTR* 2756A>G, *TYMS* 28bp repeats, *DNMT3B* -149C>T, *DNMT3B* -579G>T polymorphisms by PCR-RFLP technique (as alterations in folate metabolism have been implicated in the development of various diseases in humans, like cancer). Data were analyzed with the ANN, able to understand non-linear relationships among studied variables and to highlight through a graph the complexity of connections among them.

**Results:** Interesting correlations among gender, onset of pathology, Osserman classification and thymic pathology emerged. By selection of specific variables a correlation among thymoma, polymorphisms and Osserman classification was observed.

**Conclusions:** ANN revealed the complexity of the interconnections among factors linked to thymic pathology in MG.

#### P18.012

##### Association study of OPRM1, OPRK1 and COMT genes polymorphisms and pain perception in cancer pain Tunisian patients.

**I. Chatti<sup>1</sup>, J. B. Woillard<sup>2</sup>, I. Creveaux<sup>3</sup>, L. Ben Fatma<sup>4</sup>, S. Ben Ahmed<sup>4</sup>, A. Saad<sup>1</sup>, B. Allani<sup>5</sup>, J. Feki<sup>6</sup>, M. Gribaa<sup>1</sup>, F. Libert<sup>7</sup>;**

<sup>1</sup>Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology. Farhat Hached Hospital, Sousse, Tunisia, <sup>2</sup>INSERM UMR 850, Univ Limoges, CHU Limoges, Limoges, France, <sup>3</sup>Laboratoire de Biochimie médicale, Faculté de Médecine, Clermont-Ferrand, France, <sup>4</sup>Department of Medical Oncology, CHU Farhat Hached, Sousse, Tunisia, <sup>5</sup>Service de carcinologie médicale- Institut Salah Azaiez, Tunis, Tunisia, <sup>6</sup>Service d'oncologie médicale et service de chirurgie générale, CHU Habib Bourguiba. Université de Sfax, Sfax, Tunisia, <sup>7</sup>Laboratoire de Pharmacologie et Toxicologie, CHU G.Montpied, Clermont-Ferrand, France.

**Introduction:** Opioid therapy is a mainstay in acute and chronic pain management especially for cancer related pain. Genetic causes for inter-individual variability of the clinical response to opioids have been proved for several years. Mu opioid receptor (MOR) is the primary target of morphine for cancer pain treatment. KAPPA opioid receptor (KOR) has a functional interaction with MOR activation pathway and COMT (Cathecol-O-methyltransferase) is a key enzyme that metabolizes catecholamines in the nervous system and has been linked to substance abuse. In this study, we focused on six polymorphisms in Mu and Kappa opioid receptors and COMT genes

(OPRM1; OPRK1 and COMT respectively), and their association to morphine dose needed to decrease the pain intensity in tunisian cancerous patients. **Materials and Methods:** We collected 129 blood samples from cancerous patients under morphine treatment at different doses. DNA extraction was achieved for all patients. By Simple Probe probes on Light Cycler, we screened for the 6 SNPs in OPRM1, OPRK1 and COMT genes. Statistical analysis were performed by "R" software.

**Results:** We detected significant association of one SNP in the OPRK1 gene and bone metastasis ( $p= 0.02783$ ).

**Conclusion:** We showed, for the first time, an association between one SNP in OPRK1 and bone metastasis. No evident association of the other polymorphisms screened and the dose of morphine needed for pain relief was proved in our population.

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#### P18.013

##### Integrating transcriptomic and genomic data to identify genes involved in acute respiratory syndrome

**M. Pino-Yanes**<sup>1,2</sup>, N. Hernández-Pacheco<sup>1,3</sup>, M. Acosta-Herrera<sup>1,2,4</sup>, A. Corrales<sup>1,2</sup>, A. Barreto-Luis<sup>1</sup>, B. Guillen-Guio<sup>1</sup>, E. Zavala<sup>2,5</sup>, J. Belda<sup>6</sup>, S. Ma<sup>7</sup>, J. Villar<sup>2,4</sup>, C. Flores<sup>1,2,3</sup>;

<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain, <sup>2</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, <sup>3</sup>Applied Genomics Group (G2A), Section of Genetics, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, Tenerife, Spain, <sup>4</sup>Multidisciplinary Organ Dysfunction Evaluation Research Network (MODERN), Research Unit, Hospital Universitario Dr. Negrín, Gran Canaria, Spain, <sup>5</sup>Surgical Intensive Care Unit, Department of Anesthesia, Hospital Clinic, Barcelona, Spain, <sup>6</sup>Surgical Intensive Care Unit, Department of Anesthesiology and Intensive Care, Hospital Clínico Universitario de Valencia, Valencia, Spain, <sup>7</sup>Pulmonary & Critical Care Medicine, University of Chicago, Chicago, IL, United States.

**Introduction:** Acute respiratory distress syndrome (ARDS) is one the main causes of mortality in adults admitted to the Intensive Care Units (ICU) and it can be caused by the progression of sepsis. Many previous studies have demonstrated the contribution of genetic variants in the susceptibility and outcomes of this syndrome. In this study, we aimed to identify novel genes implicated in sepsis-derived ARDS susceptibility and/or survival.

**Materials and Methods:** We performed a prioritization of candidate genes by means of the integration of genomic data from a transcriptomic study in an animal model and from a genome-wide association study of trauma-induced ARDS in humans. From them, three single nucleotide polymorphisms (SNPs) from three different genes were selected to conduct a genetic association study. DNA samples from unrelated individuals with ARDS derived from sepsis (n=349) and from population-based controls (n=900) were genotyped using competitive allele-specific PCR assays.

**Results:** A SNP from *FLT1* gene (rs9513106) was associated with ARDS susceptibility, with an odds ratio (OR)= 0.81 for the C allele, 95% confidence interval (CI): 0.65-1.00,  $p=0.037$ . Moreover, a SNP from *ITGA1* gene (rs16880534) was associated with ICU mortality among ARDS patients (OR= 2.94 for the G allele, 95%CI: 1.46-5.94  $p=0.003$ ).

**Conclusions:** Albeit the validation of these results is ongoing, the integration of genomic data from different sources constitutes a promising method to identify new candidate genes involved in ARDS susceptibility and progression.

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#### P18.014

##### A pathway-based enrichment analysis identifies FZD6-CTHRC1 as a novel asthma susceptibility locus

**A. Barreto-Luis**<sup>1,2</sup>, M. Pino-Yanes<sup>1,3</sup>, A. Corrales<sup>1,3</sup>, M. Acosta-Herrera<sup>1,2,4</sup>, C. Gonzalez-Colino<sup>5</sup>, J. Villar<sup>2,4</sup>, P. Campo<sup>6</sup>, J. Cumplido<sup>7</sup>, J. Martinez-Tadeo<sup>5</sup>, M. Blanca<sup>8</sup>, T. Carrillo<sup>7</sup>, C. Flores<sup>1,2,3</sup>;

<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain,

<sup>2</sup>Applied Genomics Group (G2A), Section of Genetics, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, Tenerife, Spain,

<sup>3</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain,

<sup>4</sup>Multidisciplinary Organ Dysfunction Evaluation Research Network (MODERN), Research Unit, Hospital Universitario Dr. Negrín, Gran Canaria, Spain, <sup>5</sup>Allergy Unit, Hospital Universitario N.S. de Candelaria, Tenerife, Spain, <sup>6</sup>U.G.C. Allergy, Regional University Hospital of Málaga-IBIMA, Málaga, Spain, <sup>7</sup>Allergy Unit, Hospital Universitario Dr. Negrín, Gran Canaria, Spain.

**Introduction:** Accumulated association evidence in genes related by biological pathways can reveal novel asthma genes.

**Materials and Methods:** Based on summary data from our genome-wide association study (GWAS) of asthma, we identified significant biological

pathways using a gene-set enrichment analysis. We then mapped all tested single nucleotide polymorphisms (SNPs) on the genes contributing to significant pathways, and prioritized those with a disproportionate number of nominal significant associations for replication studies. Replication was performed for selected SNPs from those genes in independent case-control samples (n=1,793) using logistic regressions, and results were meta-analyzed with those from the GWAS.

**Results:** Two biological processes were significantly enriched: the cytokine-cytokine receptor interaction ( $p=0.002$ ) and the WNT signalling pathway ( $p=0.012$ ). Out of the 417 genes interacting in these two pathways, nine showed an excess of nominal associations, including two firm asthma susceptibility genes (SMAD3 and CSF2-IL3) and seven other novel candidate genes. From the latter, 14 SNPs were followed-up and one of them located in the FZD6-CTHRC1 locus was replicated ( $p=9.90 \times 10^{-4}$ ), which had a consistent direction of effects with the GWAS findings (meta-analyzed odds ratio=1.49;  $p=5.87 \times 10^{-6}$ ).

**Conclusions:** This study revealed the importance of two biological pathways in asthma pathogenesis and evidenced that the FZD6-CTHRC1 locus contributes to asthma susceptibility.

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## P18.015

### Genome-wide interaction study of time-to-asthma onset with early environmental tobacco smoke exposure

C. Sarnowski<sup>1,2</sup>, M. H. Dizier<sup>1,2</sup>, R. Granell<sup>3</sup>, D. Jarvis<sup>4,5</sup>, M. J. Ege<sup>6,7</sup>, C. Laprise<sup>8</sup>, P. Sugier<sup>1,9</sup>, P. Margaritte-Jeannin<sup>1,2</sup>, W. O. C. Cookson<sup>10</sup>, M. Moffatt<sup>10</sup>, M. Lathrop<sup>11</sup>, I. Pin<sup>12,13,14</sup>, E. Von Mutius<sup>8,7</sup>, V. Siroux<sup>12,13,15</sup>, J. Henderson<sup>3</sup>, M. Kogevinas<sup>16,17,18</sup>, F. Demenais<sup>1,2</sup>, E. Bouzigon<sup>12</sup>, <sup>1</sup>INSERM UMR-946, Paris, France, <sup>2</sup>Université Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris, France, <sup>3</sup>School of Social and Community Medicine, Bristol, United Kingdom, <sup>4</sup>Respiratory Epidemiology, Occupational Medicine and Public Health, National Heart and Lung Institute, Imperial College, London, United Kingdom, <sup>5</sup>MRC-PHE Centre for Environment & Health, London, United Kingdom, <sup>6</sup>Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany, <sup>7</sup>Comprehensive Pneumology Center Munich (CPC-M), German Center for Lung Research, Munich, Germany, <sup>8</sup>Département des sciences fondamentales, Université du Québec à Chicoutimi, Saguenay, QC, Canada, <sup>9</sup>Université Pierre et Marie Curie, Paris, France, <sup>10</sup>National Heart Lung Institute, Imperial College London, London, United Kingdom, <sup>11</sup>McGill University and Génome Québec Innovation Centre, Montreal, QC, Canada, <sup>12</sup>Univ. Grenoble Alpes, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, GRENOBLE, France, <sup>13</sup>Inserm, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France, <sup>14</sup>CHU de Grenoble, Pediatrics, Grenoble, France, <sup>15</sup>CHU de Grenoble, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France, <sup>16</sup>Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain, <sup>17</sup>CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain, <sup>18</sup>IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain.

**Background:** The number of genetic factors identified for asthma remains limited. The study of gene-by-environment interactions may facilitate the discovery of new genes. Early environmental tobacco smoke (ETS) exposure (in utero or during infancy) is a known risk factor for childhood-onset and late-onset asthma.

**Objective:** Our goal was to identify genetic variants interacting with early ETS exposure that influence time-to-asthma onset (TAO).

**Methods:** We conducted a large-scale meta-analysis of five genome-wide interaction studies (GEWIS) of TAO (totaling 3,643 exposed (ETS+) and 5,275 non-exposed (ETS-) individuals of European ancestry) using survival analysis methodologies. Two tests were performed: 1) a joint test of SNP effect and GxETS interaction and 2) a test of GxETS interaction alone.

**Results:** While the joint test confirmed two asthma regions (9p24 & 17q12-q21) interacting with ETS on TAO at the genome-wide significant level ( $P < 5 \times 10^{-8}$ ), the interaction test revealed three new loci: 13q21, 16p13 and 19q13 ( $6.7 \times 10^{-7} < P < 10^{-6}$ ). Further analysis of the 9p24 and 17q12-q21 loci stratified on asthma age-of-onset (before and after six years) confirmed the known ETSx17q12-q21 interaction in childhood-onset asthma and evidenced a complex effect of 9p24 top SNP on asthma risk with: 1) the strongest effect in ETS+ early-onset subjects (HR [CI]=1.41 [1.25-1.58]), 2) an intermediate effect in both ETS- early-onset (HR=1.23 [1.12-1.34]) and ETS+ late-onset subjects (HR=1.26 [1.14-1.39]) and 3) no effect in ETS- late-onset subjects ( $P=0.38$ ).

**Conclusion:** This study suggests an important role of early ETS exposure and 9p24 genetic variants in asthma age-of-onset.

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## P18.016

### Genetic susceptibility loci for coronary artery disease and large artery stroke are associated with human atherosclerotic plaque characteristics

S. W. van der Laan<sup>1</sup>, M. A. Siemelink<sup>1</sup>, S. Haitjema<sup>1</sup>, H. Foroughi Asl<sup>2</sup>, M. Mokry<sup>1</sup>, J. van Setten<sup>1</sup>, R. Malik<sup>3</sup>, M. Dichgans<sup>3</sup>, B. B. Worrall<sup>4</sup>, N. J. Samani<sup>5,6</sup>, H. Schunkert<sup>7</sup>, J. Erdmann<sup>7</sup>, U. Hedin<sup>8</sup>, G. Paulsson-Berne<sup>9</sup>, J. L. M. Björksgren<sup>9</sup>, G. de Borst<sup>1</sup>, F. W. Asselbergs<sup>1,8,9</sup>, H. M. de Ruijter<sup>1</sup>, P. I. W. de Bakker<sup>1</sup>, G. Pasterkamp<sup>1</sup>,

<sup>1</sup>UMC Utrecht, Utrecht, Netherlands, <sup>2</sup>Karolinska Institutet, Stockholm, Sweden,

<sup>3</sup>Institute for Stroke and Dementia Research, Munich, Germany, <sup>4</sup>University of Virginia, Charlottesville, VA, United States, <sup>5</sup>University of Leicester, Leicester, United Kingdom,

<sup>6</sup>Glenfield Hospital, Leicester, United Kingdom, <sup>7</sup>Deutsches Herzzentrum München, Munich, Germany, <sup>8</sup>Durrer Center for Cardiogenetic Research, Utrecht, Netherlands,

<sup>9</sup>University College London, London, United Kingdom.

**Background:** Tens of genetic susceptibility loci associate with large artery ischemic stroke (LAS) and coronary artery disease (CAD), but deciphering their underlying mechanisms to identify putative therapeutic targets is challenging. Atherosclerosis is cardinal to cardiovascular diseases (CVD) and histological studies have identified plaque characteristics that associate with clinical outcome. To what extent common variation associated with CVD relates to these characteristics remains unknown. We studied the impact of sequence variation on plaque characteristics, and tissue-specific gene expression by combining data from independent biobanks comprising patients with clinically significant arterial stenosis.

**Methods:** We genotyped 1,439 patients from Athero-Express, 127 patients from BiKE, and 109 patients from STAGE. We tested 61 CVD susceptibility loci for association to seven plaque characteristics, and gene expression using regression modeling, correcting for age, sex, 10 principal components, and study specific covariates.

**Results:** We report a  $\sim 5.2$ -fold enrichment of CAD variants associated with plaque characteristics ( $p = 3.6 \times 10^{-8}$ , 16 out of 61 variants). The CAD risk reducing alleles of rs12539895 and a nearby deletion (chr7:106,901,393 TG > T) on 7q22 associated with less fat in plaques ( $p < 5.1 \times 10^{-6}$ ), and lower circulating LDL levels. Circularized chromosome conformation capture in monocytes revealed many regional genes physically interacting with rs12539895. Additional analyses revealed tissue-specific effects on HBP1, COG5, and GPR22 expression, further prioritizing the list of 11 regional genes for future studies.

**Conclusion:** Our study supports the view that genetic loci conferring susceptibility to CAD and LAS, play a role in the underlying pathophysiology of the atherosclerotic plaque.

## P18.017

### Bardet Biedl syndrome shows a distinctive genetic epidemiology in Iran: targeted next generation sequencing revealed 15 novel mutations in 6 genes previously reported for the disease

M. Keramatipour<sup>1</sup>, Z. Golchahre, D. Kooshavar, A. Karamzade, M. Razipour, M. Entezam, M. Saberi; <sup>1</sup>Tehran University of Medical Sciences, Department of Medical Genetics, Tehran, Iran, Islamic Republic of.

Bardet Biedl Syndrome (BBS) is a rare autosomal recessive (AR) and genetically heterogeneous ciliopathy characterized by retinitis pigmentosa, obesity, polydactyly, learning disabilities, renal involvement, and genital abnormalities. So far 21 genes have been identified to cause BBS. In this study 18 unrelated patients with clinical diagnosis of BBS were investigated by target region capturing of 18 BBS-related genes (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, MKS1, CEP290, SDCCAG8, WDPCP, TMEM67, LZTFL1) followed by next generation sequencing (NGS). NGS results were then validated by Sanger sequencing. The genetic diagnosis was achieved in 15 patients (83.33%) including 15 novel and 1 previously reported variants in 6 genes (one case was compound heterozygote). The novel variants were absent in population and disease-specific databases, were predicted to be disease causing by multiple *in silico* predictive tools, and changed nucleotides were evolutionarily well conserved. All variants were shown to be segregating in the family. Based on the American College of Medical Genetics and Genomics (ACMG) guidelines for the interpretation of sequence variants, they are classified as pathogenic/likely pathogenic variants. The most commonly involved genes were BBS9 and BBS12, each with a frequency of 26.66%, followed by TTC8, BBS10, BBS7, with a frequency of 13.33% each, and MKKS with 6.66% frequency. Detailed data will be presented at the meeting. Due to distinctive spectrum of causative genes with mostly novel mutations, it seems that the genetic epidemiology of the disease in Iran is different from that of other reported populations.

## P18.018

### Genome-wide association study of bitter taste perception of quinine in an isolate population of Croatia

A. Relja<sup>1</sup>, I. Patarčić<sup>1</sup>, A. Miljković<sup>1</sup>, C. Hayward<sup>2</sup>, I. Rudan<sup>3</sup>, I. Kolčić<sup>1</sup>;

<sup>1</sup>Medical School, University of Split, Split, Croatia, <sup>2</sup>Institute for Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>3</sup>Usher Institute, University of Edinburgh, Edinburgh, United Kingdom.

**Aim:** Taste perception plays an important role in the diet composition, which is the leading lifestyle risk factor for cardiovascular diseases and cancer. The aim of this study was to investigate genetic background of bitter taste perception.

**Materials and methods:** Subjects from the 10,001 Dalmatians cohort were involved in the bitter taste recognition threshold measurement, based on water solution of quinine, starting from 1E-06 M and increasing by 0.22 log value. A GWAS meta-analysis was performed using 317.500 SNPs in a sample of 936 individuals from the Island of Korcula and Vis, and the City of Split, Croatia, controlling for age, gender and kinship.

**Results:** Bitter taste threshold was strongly associated with 8 SNPs from PRH1-PRR4, PRH1, TAS2R19 and TAS2R20 gene, with P values between 1.5E-11 to 8.8E-39, belonging to the following SNPs: rs4763602, rs10772420, rs12226919, rs12226920, rs10845279, rs7307031, rs10845289 and rs10492102. Additionally, we detected marginally suggestive SNP at chromosome 6, belonging to ADGRF5 gene (rs1004018; P= 4.4E-07).

**Conclusion:** While the finding of the PRH1-PRR4, PRH1, TAS2R19 and TAS2R20 genes confirms the previous studies, the ADGRF5 gene is reported for the first time, suggesting even greater complexity of the genetic basis of bitter taste perception. These results could be used in numerous applications, including personalized dietary planning, fitness and other health-related utilization. Further refinement of these results in the imputed data and increased sample sizes is under way.

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## P18.019

### Rare variant association tests for survival outcomes in population and family structured studies

Y. Chiou<sup>1</sup>, L. Chien<sup>2</sup>, D. Bowden<sup>3</sup>;

<sup>1</sup>National Health Research Institutes, Zhunan, Taiwan, <sup>2</sup>Academia Sinica, Taipei, Taiwan,

<sup>3</sup>Wake Forest School of Medicine, Winston-Salem, NC, United States.

Functional variants are likely to be aggregated in family studies enriched with affected members, and this aggregation increases the statistical power for rare variant detection. In addition to the identification of associations with qualitative and quantitative traits under a generalized linear model framework, associations of rare variants with time-to-event traits are of growing interest. However, very limited approaches are available for the analysis of rare variant associations with time-to-event traits in family or hybrid designs. Therefore, we developed novel pedigree-based burden and kernel association tests for family time-to-event outcomes with right censoring, and used Cox proportion hazard models to relate a time-to-event trait with rare variants. We describe how to employ Cox models in commonly used pedigree-based burden and kernel tests. Furthermore, we demonstrate the robustness of our proposed tests when the proportional hazard assumption is violated. Statistical inferences can be made through asymptotic distributions of the proposed tests, permitting their application to large-scale whole-genome sequencing data. The proposed tests are appropriate for practical use under a wide range of misspecified Cox models, as well as for hybrid population- and pedigree-based designs. Extensive simulation studies were conducted to compare the proposed tests with existing gene-based rare variant association tests for family survival outcomes. We applied the proposed tests to the exome chip data from the Diabetes Heart Study to identify variants associated with age at onset of type 2 diabetes.

## P18.020

### Genetic structure and demographic history of the Caucasus: insights from whole-genome sequences

M. Mezzavilla<sup>1,2</sup>, M. Haber<sup>2</sup>, Q. Ayub<sup>2</sup>, Y. Xue<sup>2</sup>, P. Gasparini<sup>1,3</sup>, C. Tyler-Smith<sup>2</sup>;

<sup>1</sup>Sidra Medical and Research Center, Doha, Qatar, <sup>2</sup>The Wellcome Trust Sanger Institute, Wellcome Genome Campus, Cambridge, United Kingdom, <sup>3</sup>University of Trieste, Trieste, Italy.

Archaeological findings suggest that modern humans have inhabited the Caucasus since Paleolithic times but little is known about their origin and how they have interacted with neighboring populations. Previous genetic studies of present-day populations from the Steppe suggested the Caucasus

acted as a barrier, leading to genetic discontinuity between the Caucasus and the East European Plain. Furthermore, geography, ethnicity, and language are also expected to influence the mating patterns of the populations inhabiting the Caucasus and thus create genetic structure within this region. Here we use whole-genome genotyping and sequencing data to fully understand the genetic diversity of the Caucasus and its role in the demographic process that shaped modern Eurasian genomes.

Combining data from 12 high-coverage whole-genome sequences, four each from Georgia, Armenia, and Azerbaijan with SNP-genotype data from more than 250 individuals from the same populations, we present one of the most comprehensive datasets for this region. Principal component analyses, followed by model-based clustering, reveal previously unreported structure among the Caucasus populations. In particular, we discovered three different clusters among Georgians and two in Azerbaijan, in addition to different ancestral proportions in these clusters. MSMC analysis of the whole genomes shows Caucasus populations have diverged in the last 20,000 years from other Eurasians and have experienced different demographic changes in recent times

We thus provide a detailed analysis of the past and present demography of the Caucasus which will be useful for understanding the genetic diversity of this region and the relationship with European populations.

## P18.021

### A novel mutation in MTMR2 gene causing CMT4B1; A founder effect in Ashkenazi Jews

T. Abdalla<sup>1</sup>, A. Peleg<sup>1</sup>, O. Sadeh<sup>1</sup>, K. Badarneh<sup>1</sup>, G. Larom-Khan<sup>1</sup>, F. Fares<sup>2</sup>;

<sup>1</sup>Department of Human Genetics, Carmel Medical Center, Haifa, Israel, <sup>2</sup>Department of Human Biology, University of Haifa, Haifa, Israel.

Charcot-Marie-Tooth type 4B1 (CMT4B1) is a rare type of the CMT group caused by mutations in the Myotubularin-related protein 2 (MTMR2) gene. A novel frame-shift mutation was found in an Ashkenazi Jewish patient, diagnosed with CMT in childhood. Whole exome sequence indicated that the patient is homozygous for the mutation c.1877\_1878insAGAG in the MTMR2 gene. To our knowledge, this mutation was never reported. Both parents are healthy, no consanguinity reported, and DNA sequencing analysis indicated that both are heterozygote for this mutation. Such surprising finding, made us suspect a founder mutation in the Ashkenazi population.

We conducted a research aiming to examine the prevalence of this novel mutation in Ashkenazi Jews. If it is indeed a common mutation, it is worth considering including it in the genetic screen offered for this population in Israel.

We assumed that if the prevalence of this variant was 1:100 among Ashkenazi Jews, then the study should include at least 400 samples, in order to detect at least one heterozygous object (confidence interval for 0% is <1%). PCR analysis followed by DNA sequencing was performed, on a total of 450 samples. No heterozygous were found, hence the prevalence of this novel mutation in Ashkenazi Jews is less than 1:100. Another explanation for this finding is an unknown or unreported consanguinity between parents.

Therefore, at this stage, based on our results, it is not recommended to include this novel mutation as a part of the genetic screening for Ashkenazi Jews.

## P18.023

### Common genetic basis between chronic venous disease and venous thrombosis

A. Martinez-Perez<sup>1</sup>, J. Romero<sup>2</sup>, A. Ziyatdinov<sup>1</sup>, A. Viñuela<sup>3</sup>, M. Sabater-Lleal<sup>4</sup>, A. Hamsten<sup>4</sup>, E. Dermitzakis<sup>3</sup>, J. Souto<sup>5</sup>, J. Escudero<sup>2</sup>, A. Buil<sup>6</sup>, J. Soria<sup>1</sup>;

<sup>1</sup>Unitat de Genòmica de Malalties (IIB-Santpau), Barcelona, Spain, <sup>2</sup>Servicio de Angiología, Cirugía Vascular y Endovascular. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>University of Geneva, Geneva, Switzerland, <sup>4</sup>Cardiovascular Medicine Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, <sup>5</sup>Unitat de Trombosis i Hemostàsia. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,

<sup>6</sup>Institut of Biological Psychiatry, Mental Health Snc. Hans, Roskilde, Denmark.

**Introduction:** Chronic Venous Disease (CVD) is a common multifactorial and complex disease characterized by an abnormal reflux of blood in veins. Despite the high prevalence of CVD, the hereditary component is still unknown. The purpose of this study is to quantify the genetic basis of the CVD and its relationship to venous thromboembolism (VTE).

**Methods:** The sample consisted of 895 individuals (60 patients with CVD) belonging to 35 extended pedigrees in the GAIT2 Project. Patients were diagnosed by venous Doppler ultrasound technique following the guidelines of the American Venous Forum. We correlated this disease with other thromboembolic diseases and 86 hemostatic phenotypes using variance component in SOLAR. The genome-wide association study (GWAS) was performed with 9M SNPs using a mixed model and the analysis of association of RNAseq ex-

pression with CVD was performed using bivariate mixed models in Solarium. Results: Heritability of the CVD was 76%. More interestingly, we found a highly significant genetic correlation with VTE, 0.76. Only suggestive signals were found from the GWAS and RNAseq studies.

Conclusions: To our knowledge this is the most extensive study (clinical and biological phenotypes, GWAS and RNAseq data) performed to date to unravel the genetic components of CVD and other related venous diseases. Despite this huge amount of data we did not find any clear signal to explain the high heritability observed and the genetic correlation between CVD and VTE. These results emphasized the high complexity of the genetic basis underlying this disease.

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#### P18.024

##### Evolutionary ancient human genes accumulate intronic deletions

M. Rigau, D. Juan, A. Valencia, D. Rico;

Spanish National Cancer Research Centre, CNIO, Madrid, Spain.

Genomic copy number variants (CNVs) are thought to account for an important part of human variability. The number, location and type of CNVs that are annotated in the different studies depend largely on the techniques and algorithms used for their detection. We have compared five of the most comprehensive high resolution CNV maps published in 2015 (Handsaker et al.; Zarrei et al.; Sudmant et al., Science; Sudmant et al., Nature; Abzyov et al.) and evaluated the potential impact of the CNVs on protein-coding genes by classifying them in different gene ages. In all maps, we see that CNVs that encompass whole genes (thus influencing their gene dosage) are significantly enriched in Primate-specific genes and significantly depleted in more ancient genes, confirming and extending our previous results (Juan et al., *Biology Open*, 2013).

The higher resolution of the integrated experiments allows us to uncover that when CNVs are overlapping with exons, they accumulate in recent genes, a similar pattern to that of whole-gene CNVs. However, partial CNVs located within introns are over-represented in ancient genes. More precisely, genes born before the ancestor of human and bony fish show an excess of intronic deletions in current human populations. We will present our ongoing analyses characterising the genomic, epigenomic and functional characteristics of the ancient genes showing these intronic deletions. Taken together, our results show how the precise mapping of CNVs in combination with phylogenetic information allows us to understand the differential impact of duplications and deletions in protein-coding regions.

#### P18.025

##### Environmental exposures modify gene expression in colon mucosa

D. Aguilar<sup>1</sup>, M. Alonso<sup>1</sup>, A. Diez<sup>1</sup>, E. Guinó<sup>1</sup>, I. Padrol<sup>1</sup>, F. Rodriguez-Moranta<sup>2</sup>, M. Barenys<sup>3</sup>, G. Ibañez-Sanz<sup>1</sup>, G. Casey<sup>4</sup>, V. Moreno<sup>1,5</sup>;

<sup>1</sup>Catalan Institute of Oncology, IDIBELL and CIBERESP, Hospital Llobregat, Spain,

<sup>2</sup>Bellvitge University Hospital, Hospital Llobregat, Spain, <sup>3</sup>Hospital de Viladecans, Viladecans, Spain, <sup>4</sup>University Southern California, Los Angeles, CA, United States,

<sup>5</sup>University of Barcelona, Barcelona, Spain.

Introduction: Colorectal cancer (CRC) is a complex disease with both genetic and environmental risk factors contributing to susceptibility. The analysis of gene-environment interactions (GxE) can identify new susceptibility loci but statistical power is reduced in part because agnostic searches require adjustment for millions of tests. To reduce this burden and improve power in a GxE analysis, one option is to incorporate functional genomics data to prioritize the candidate SNPs.

Materials and methods: We are generating RNA-seq data from 300 normal colon biopsies from voluntary subjects undergoing colonoscopy within a screening program. Detailed environmental and lifestyle risk factor information is requested by personal interview. Here we focus on the analysis of the association between gene expression and environmental variables (eQTL analyses will follow). Linear models have been used, adjusted for age, sex and education. Empirical Bayes adjustments and false discovery rates are calculated to reduce false-positive results. Gene-Set Enrichment Analysis has been also performed to identify pathways modified by each environmental exposure.

Results: To date, 145 samples have been fully analyzed and preliminary results indicate that significant associations can be identified for genes with expression correlated to red meat, processed meat, vegetables, fruits, alcohol, smoking, physical exercise, aspirin and BMI.

Conclusions: Environmental variables known to be risk factors for CRC modify the expression of specific genes in the normal colon mucosa. Genetic variation in these genes may provide further insight into the complex etiology of CRC.

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#### P18.026

##### A novel gene-gene interaction test for rare variants in complex disease studies using case-control samples.

C. Kang, R. Chung;

Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan.

Introduction: Gene-gene interaction can play an important role in complex disease etiology. With the advancement of next-generation sequencing, many rare variants have been discovered. However, current gene-gene interaction tests are mainly developed for common variants. Therefore, a gene-gene interaction test accounting for rare variants is desirable.

Materials and Methods: We developed a powerful gene-gene interaction test, which includes several steps: (1) for a pair of genes, odds ratios of interaction for all pairs of rare SNPs between the two genes are calculated; (2) the SNP pairs are ranked by the odds ratios, and the pairs with rank in the highest and lowest 2.5% are selected; (3) calculate the overall genotype counts for the selected pairs. The interaction statistic based on odds ratios (IOR) or goodness-of-fit (IGOF) is calculated using the genotype counts. (4) Permutations are used to calculate the p-value.

Results: We used simulations to evaluate the type I error rates and compare the power of the proposed tests with another interaction test for rare variants (SPAr). Our simulation results suggest that the IOR and IGOF have appropriate type I error rates. Moreover, IGOF is generally more powerful than IOR, while both tests have higher power than SPAr.

Conclusions: In conclusion, the IOR and IGOF will be very useful to detect gene-gene interactions for rare variants. We implemented the IOR and IGOF into an efficient software with C++ incorporating threads and MPI. This study was supported by a grant from the Ministry of Science and Technology in Taiwan (MOST 104-2221-E-400-004-MY2).

#### P18.027

##### Characterization of CNVs not previously described, among Valencian population: local reference database

C. Monzó Cataluña<sup>1</sup>, P. Marín Reina<sup>2</sup>, I. Quintela García<sup>3</sup>, D. Albuquerque do Santos<sup>4</sup>, L. Gandía Artigues<sup>1</sup>, C. Guzmán Luján<sup>1</sup>, G. Marcaida Benito<sup>1</sup>, M. Aleu Pérez-Gramunt<sup>2</sup>, R. Rodríguez López<sup>1</sup>;

<sup>1</sup>Genetics Laboratory, General Hospital of Valencia, Valencia, Spain, <sup>2</sup>Department of Pediatrics, General Hospital of Valencia, Valencia, Spain, <sup>3</sup>National Genotyping Centre, NODE, Santiago de Compostela, Spain, <sup>4</sup>Research Center for Anthropology and Health (CIAS), Coimbra, Portugal.

Introduction: The diagnostic algorithm for intellectual disability which was described in the Consensus for the Implementation of Arrays 2012, has generated the massive identification of new copy number variations (CNVs). Their functional assessment requires a comparison with the described CNVs and with our population scope.

Patients and Methods: We analyzed the CNVs identified in 28 boys and 22 girls, who were studied for having developed non-recognized syndromic phenotypes, with Valencian ancestor. The genomic array using SNPs/CNVs, CytoScan® HD Affymetrix, was applied. The obtained raw data, were analyzed with Chromosome Analysis Suite (Chas) software. The exact location of each CNV, was compared with overlapping variants, which were described in the CNVs data bases.

Results: 16 not described CNVs appeared. Their location overlapped with similar CNVs referred in ISCA as pathogenic. 7 patients, which result array was negative, carried one of these 7 CNVs (92 to 2 kbp); local origins were corroborated. The other 9 CNVs (16 to 4 kbp) are recurrently found in the series. Two patients carried the LOH fragments of 33.9 and 30.8 Mb, respectively.

Conclusions: The percentage of solved cases was similar to that proposed in the Consensus (20%). 7 of the 11 resolved cases (63.6%) carried cryptic causal alterations (<4 Mb). Building databases of CNVs with undetermined significance, from individuals of population level, was essential to the determination of its deleterious and informativeness capacity. Its existence repeatedly did not rule out its possible relationship with the clinical phenotype; wide association studies are essential for their proper assessment.

#### P18.028

##### Fine-scale genetic population structure and genetic risk for coronary artery disease in Finland

S. Kermiinen<sup>1</sup>, I. Surakka<sup>1</sup>, A. Sarin<sup>1</sup>, E. Tikkainen<sup>1</sup>, G. Hellenthal<sup>2</sup>, A. Havulinna<sup>3</sup>, M. Perola<sup>2</sup>, V. Salomaa<sup>3</sup>, S. Ripatti<sup>1</sup>, M. Pirinen<sup>1</sup>;

<sup>1</sup>Institute for Molecular Medicine Finland FIMM, University of Helsinki, Helsinki, Finland,

<sup>2</sup>University College London, London, United Kingdom, <sup>3</sup>National Institute of Health and Welfare, Helsinki, Finland.

Introduction: Previous studies have reported genetic differences between South-West (SW) and North-East (NE) Finland and a similar division can be seen, e.g., in incidence of coronary artery disease (CAD). We refine the

current knowledge of the genetic structure in Finland and study whether it explains regional differences in incidence of CAD.

**Material and Methods:** We apply recent haplotype-based methods ChromoPainter and fineSTRUCTURE to 2376 such individuals from the FINRISK Study survey of 1997 for whom both parents were born close to each other. We generate two CAD genetic risk scores: (1) "Lead-SNP" score using 153 independent lead SNPs and (2) "Polygenic" score using 49,000 LD-pruned SNPs with P-value below 0.05 from CARDIoGRAMplusC4D Consortium.

**Results:** We show that the genetic borderline between NE and SW Finland follows closely the historic border from 1323 with relatively little uncertainty. Among the genetically identified subpopulations, incidence of CAD in the NE region of Kainuu is 1.82 times as high as in SW Finland and our "Lead-SNP" score for CAD yields an odds ratio of 1.11 (p=0.025) between Kainuu and SW Finland. The distribution of our "Polygenic" score shows relatively high risk for CAD in East and low risk in SW, which further reflects the known incidence of the disease.

**Conclusions:** Haplotype-based methods reveal new details describing Finnish population structure. Geographic distributions of genetic risk scores and incidence of CAD have similarities suggesting that some of the regional differences in risk of CAD are genetic.

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#### P18.029

##### Genetic Association of Coronary Artery Disease in the Singaporean Chinese population: Replication of known loci at CDKN2A/2B, PHACTR1, BCAS3, ABO and VAMP5/VAMP8/GGCX

*Y. Han<sup>1</sup>, R. Dorajoo<sup>2</sup>, X. Chang<sup>1</sup>, C. Khor<sup>2</sup>, J. Yuan<sup>3</sup>, W. Koh<sup>4,5</sup>, E. Tai<sup>4,6</sup>, J. Liu<sup>2,4</sup>, R. M. van Dam<sup>4,6</sup>, Y. Friedlander<sup>7</sup>, C. Heng<sup>1</sup>;*

<sup>1</sup>Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore; and Khoo Teck Puat - National University Children's Medical Institute, National University Health System, Singapore, Singapore, <sup>2</sup>Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore,

<sup>3</sup>Department of Epidemiology, Graduate School of Public Health; and University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA, United States,

<sup>4</sup>Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore, <sup>5</sup>Duke-NUS Graduate Medical School, Singapore, Singapore, <sup>6</sup>Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Singapore, Singapore, <sup>7</sup>School of Public Health and Community Medicine, Hebrew University of Jerusalem, Jerusalem, Israel.

**Introduction:** Recent genome-wide association studies have identified 56 loci associated with coronary artery disease (CAD) among predominantly European populations. However, their relevance to Asian populations is largely unknown.

**Methods:** We utilized GWAS data (1,147 CAD cases and 3,451 controls) from two Singaporean Chinese studies. Logistic regression analyses were carried out in each dataset and subsequently meta-analyzed. We further tested for involvement of known canonical pathways for loci that had significant associations in our study using Ingenuity Pathway Analysis.

**Results:** We detected associations at five index variants at the CDKN2A/2B, PHACTR1, BCAS3, ABO and VAMP5/VAMP8/GGCX loci (P-values 6.37x10<sup>-4</sup> - 4.53x10<sup>-2</sup>). At four additional loci (in or near LPL, REST/NOA1, COL4A1/2 and ZEB2 genes), we also detected modest associations (meta-analysis nominal P-values ranging from 8.65 x 10<sup>-3</sup> - 4.46 x 10<sup>-2</sup>) for regional SNPs that were in at least moderate LD with previously reported index SNPs ( $r^2 > 0.4$  in 1000G ASN populations). Six pathways, including cell-cycle regulation pathways, glioma signaling and adipogenesis pathways (P-value 0.034 - 0.043) were identified.

**Conclusions:** We replicated the associations of at least 5 CAD-associated index SNPs among the Singaporean Chinese population. Chinese population has some common variants predisposing to CAD as Western population.

**Sources of funding:** This research was supported by the HUJ-CREATE Programme of the National Research Foundation, Singapore (Project Number 370062002). The Singapore Chinese Health Study was supported by the U.S. National Institutes of Health Grant Numbers R01CA144034 and UM1 CA182876, and by the Singapore National Medical Research Council Grant Number 1270/2010.

#### P18.030

##### Utility of genetic and non-genetic risk factors in predicting coronary heart disease in the Singaporean Chinese

*X. Chang<sup>1</sup>, R. Dorajoo<sup>2</sup>, Y. Han<sup>1</sup>, C. Khor<sup>2</sup>, R. van Dam<sup>3</sup>, J. Yuan<sup>4</sup>, W. Koh<sup>3,5</sup>, J. Liu<sup>2,3</sup>, D. Goh<sup>1</sup>, A. Salim<sup>6</sup>, Y. Friedlander<sup>7</sup>, C. Heng<sup>1</sup>;*

<sup>1</sup>Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore; and Khoo Teck Puat - National University Children's Medical Institute,

<sup>2</sup>National University Health System, Singapore, Singapore, <sup>3</sup>Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore, <sup>4</sup>Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore, Singapore, <sup>5</sup>Department of Epidemiology, Graduate School of Public Health; and University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA, United States, <sup>6</sup>Duke-NUS Graduate Medical School Singapore, Singapore, Singapore, <sup>7</sup>Department of Mathematics and Statistics, La Trobe University, Melbourne, Australia, <sup>7</sup>School of Public Health and Community Medicine, Hebrew University of Jerusalem, Jerusalem, Israel.

**Introduction:** Although numerous phenotype based equations for predicting risk of 'hard' coronary heart disease (CHD) are available, limited data exist regarding the utility of genetic information for such risk prediction in the Singaporean Chinese.

**Methods:** We conducted a case-control study nested within the Singapore Chinese Health Study (SCHS). A total of 836 males (267 cases) and 470 females (128 cases) were included. All the SNPs included were previously reported in genome-wide association studies (GWAS) and were associated with CHD or its risk factors. Cox proportional hazards models with adjustments for age and gender were used to evaluate each SNP. Those associated with p values less than 0.10 were used to build the Genetic Risk Score (GRS). Three different base models were used - M1: Recalibrated Adult Treatment Panel III (ATPIII) Model; M2: Model built on traditional risk factors used in ATPIII with their coefficients estimated using SCHS data and M3:M2+hsCRP+creatinine.

**Results:** The GRS was significantly associated with CHD. The inclusion of GRS in the prediction models were shown to improve discrimination, with c-statistics reaching between 0.750-0.758 as compared to 0.662-0.697 obtained from base models for males and between 0.827-0.835 as compared to 0.773-0.780 for females. Higher accuracies were also observed in terms of overall net reclassification improvement (NRI) index across all three models (Male:19.0%-20.4%, p<0.001; Females:14.0%-21.8%, p<0.001). The best performing model is M3.

**Conclusion:** The GRS is an independent predictor for the risk of incident 'hard' CHD in addition to the conventional risk factors and new biomarkers.

#### P18.031

##### Constitutional chromosome abnormalities among patients referred for blood karyotype analysis: a 10-year study in a Brazilian Center

*R. Sitnik<sup>1</sup>, R. M. Minillo<sup>1</sup>, E. D. R. P. Velloso<sup>1</sup>, R. K. Kishimoto<sup>1</sup>, C. I. E. C. Fabris<sup>1</sup>, J. R. R. Pinho<sup>1</sup>, C. L. P. Mangueira<sup>1</sup>, A. B. A. Perez<sup>2</sup>;*

<sup>1</sup>Hospital Israelita Albert Einstein, São Paulo, Brazil, <sup>2</sup>Federal University of São Paulo, São Paulo, Brazil.

**Introduction:** The aim of this study was to identify the profile of patients being referred for cytogenetic analysis in a Brazilian center and to determine the frequency and type of chromosomal abnormalities in our patients. **Materials and Methods:** 2417 patients (newborns to 64 years-old) were referred for a clinical genetics evaluation followed by karyotype, when indicated, between 2005 and 2015 with variable phenotypes such as mental retardation, multiple congenital malformations, clinical features of Down syndrome (DS), Turner's syndrome (TS) and Klinefelter syndrome (KS), ambiguous genitalia, infertility, amenorrhea and recurrent miscarriage.

**Results:** We could obtain karyotype from 2376 cases (98.3%). 294 (12.4%) had an abnormal karyotype. Among these, the majority was 179 females (60.8%), and there was 1 male XX (0.3%). The median age was 9 years. 63 patients (21.4%) were newborns, 51 (17.3%) were between 1 month to 1 year-old and 59 (20%) were 18 years-old or older. The commonest abnormalities were DS (57 cases (19.4%), with 3.5% mosaic and 7% robertsonian translocations) and TS (51 cases (17.3%), with 58.8% mosaic cases). We also found 19 (6.4%) KS cases (26.3% mosaic); 67 (22.7%) unbalanced rearrangements; 29 (9.9%) apparently balanced translocations, being 10 (34.4 %) relatives of index cases and 6 (2%) complex rearrangements.

**Conclusions:** We observed a lower rate of abnormalities than previous studies. Karyotyping is still an important tool for screening for malformations and developmental delay in developing countries and is also important for evaluating sexual aberrations and familial translocations.

## P18.032

**A genome-wide association scan implicates the DCHS2, RUNX2, GLI3, PAX1 and EDAR gene regions in human facial diversity**

*M. Fuentes-Guajardo<sup>1,2</sup>, K. Adhikari<sup>1</sup>, C. Gallo<sup>3</sup>, M. Bortolini<sup>4</sup>, S. Canizales-Quinteros<sup>5</sup>, F. Rothhammer<sup>6,7</sup>, G. Bedoya<sup>8</sup>, R. Gonzalez-José<sup>9</sup>, A. Ruiz-Linares<sup>10</sup>*

<sup>1</sup>University College of London, London, United Kingdom, <sup>2</sup>Universidad de Tarapaca, Arica, Chile, <sup>3</sup>Universidad Peruana Cayetano Heredia, Lima, Peru, <sup>4</sup>Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, <sup>5</sup>UNAM-Instituto Nacional de Medicina Genómica, Mexico City, Mexico, <sup>6</sup>Universidad de Tarapacá, Arica, Chile, <sup>7</sup>Universidad de Chile, Santiago, Chile, <sup>8</sup>Universidad de Antioquia, Medellín, Colombia, <sup>9</sup>Centro Nacional Patagónico, CONICET, Puerto Madryn, Argentina.

**Introduction:** Facial shape shows great variation in humans and is of considerable importance in biomedicine and forensics. Facial appearance has a strong genetic component and could have evolved to facilitate individual recognition. Although genes have been identified for various abnormal phenotypes and genome-wide studies in European populations have been conducted, the genetic basis of normal variation in human facial traits is poorly understood.

**Materials and Methods:** We conducted a Genome Wide Association Study (GWAS) using ~700,000 genome-wide markers from ~6,000 Latin American individuals. We considered fourteen ordinal facial traits examined in individual photographs. Nine of these traits were also examined in a sub-set of samples (~ 3,000) using landmarks to obtain quantitative data. Association tests used univariate linear regression with an additive genetic model considering age, sex, BMI and five genetic PCs as covariates.

**Results and Discussion:** SNPs situated in four gene regions (DCHS2, RUNX2, GLI3 and PAX1) showed associations with three ordinal traits related to nose morphology (wing breadth, root breadth, columella inclination). Quantitative analyses confirmed these associations and, in addition, detected an association of SNPs in EDAR with chin protrusion. Consistently, we characterized Edar mouse mutants and found alterations of mandible length. Finally, we replicated the reported association of nasion position with SNPs in PAX3. The genes identified have been implicated in craniofacial development, cartilage bone differentiation, malformations and the evolution of the face.

**Conclusions:** We demonstrate that human facial variation is influenced by DCHS2, RUNX2, GLI3, PAX1 and EDAR.

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## P18.033

**LHFPL5 is the gene responsible for congenital hearing impairment in Reunion Island**

*L. Jonard<sup>1</sup>, S. Marlin<sup>1</sup>, J. Lerat<sup>1</sup>, M. Jacquemont<sup>2</sup>, F. Darcel<sup>2</sup>, J. Bonnefont<sup>1</sup>, S. Gherbi<sup>1</sup>, N. Louondon<sup>1</sup>, I. Ben Aissa<sup>1</sup>, F. Saint Jalmes Digeon<sup>1</sup>*

<sup>1</sup>Necker Hospital, APHP, Paris, France, <sup>2</sup>Saint Pierre Reunion Hospital, Saint Pierre, France.

Located in the southern hemisphere, the Reunion island was isolated from the rest of the world until the first air transport. Its geography, consisting of two volcanic massifs is an obstacle to internal communications. The white Creoles, from the first core of settlers, represent 20% of the population of the island. Among them „Les Petits Blancs“ settled in „les Hauts“ during the XIX century. It is in this population that we find the highest prevalence of congenital deafness: 1.6 / 1000 against 0.8 / 1000 in metropolitan France. The genetic origins of congenital deafness are currently estimated to 80%. Two thirds of the prelingual deafness are isolated and over 40 genes implicated have been cloned to date. 90% of isolated congenital deafness are transmitted in an autosomal recessive mode. Since 2006, 20 children (from 16 families) originated from the Reunion island with bilateral profound deafness isolated received a cochlear implant in our service. 15 of them (from 12 families) presented with a similar phenotype: isolated congenital profound bilateral hearing loss, bilateral vestibular areflexia without cochleovestibular malformation nor retinal disease.

Thanks to NGS, we have involved LHFPL5 in 9/12 families. All patients carry the homozygous 185delT mutation except one family in which this deletion is combined with another variation false sense in trans. Through a study of birth records, we identified the common ancestor torque these 9 families. Alexis and Brigitte were born on the Bourbon island in 1693 and come from families arrived from the metropolitan France in 1665 and 1674.

## P18.034

**Examining the type 2 diabetes and depression link using polygenic risk scores**

*C. Kan<sup>1</sup>, N. Mullins<sup>1</sup>, D. Mehta<sup>2</sup>, J. Euesden<sup>1</sup>, M. McCarthy<sup>3</sup>, G. Breen<sup>1</sup>, Psychiatric Genetic Consortium Major Depressive Disorder Working Group, K. Ismail<sup>1</sup>, C. M. Lewis<sup>1</sup>*

<sup>1</sup>Institute of Psychiatry, Psychology and Neuroscience, London, United Kingdom, <sup>2</sup>The University of Queensland, Queensland, Australia, <sup>3</sup>Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom.

**Background:** A bidirectional association between type 2 diabetes (T2DM)

and depression has been consistently reported in epidemiological studies, suggesting that these disorders share common pathophysiological mechanisms. We examined whether polygenic risk scores for T2DM predicts depression status in the Psychiatric Genomics Consortium Major Depressive Disorder studies (PGC-MDD).

**Method:** T2DM-polygenic scores were constructed from the association summary statistics of Diabetes Genetic Replication And Meta-analysis Consortium (DIAGRAM: 34,840 cases and 114,981 controls), at nine association p-value thresholds (PT = 0.00005-0.5). Logistic regression was used to test for association between T2DM-polygenic scores and depression case/control status in 29 PGC-MDD studies (12,719 cases and 13,922 controls), adjusting for ancestry principal components and study. Cohorts included in DIAGRAM were excluded from PGC-MDD-29. Secondary analyses examined the interaction of T2DM-polygenic risk scores with i) sex and ii) Body Mass Index (BMI).

**Results:** T2DM-polygenic scores did not predict depression case status at any PT threshold, and no interaction between T2DM-polygenic scores and BMI was detected. A significant interaction between T2DM-polygenic scores and sex in predicting depression case status was found (p=0.00002, R<sup>2</sup> explained=0.087% at PT=0.001), which exceeds the Bonferroni correction threshold for 9 PT thresholds and 5 analyses (p=0.001). Stratifying by sex showed that T2DM-polygenic scores predicted depression case status in males (p=0.017, R<sup>2</sup> explained=0.067%, OR: 1.06 (1.01-1.10) at PT=0.001) but not in females (p=0.441).

**Conclusion:** This study provides the first evidence that the epidemiological association between T2DM and depression may have a genetic underpinning, particularly in males.

## P18.035

**The Irish DNA Atlas - a Study of Genetic Diversity in Ireland**

*E. H. Gilbert<sup>1</sup>, S. O'Reilly<sup>2</sup>, M. Merrigan<sup>2</sup>, D. McGettigan<sup>2</sup>, J. F. Wilson<sup>3</sup>, S. Ennis<sup>4</sup>, G. L. Cavalleri<sup>1</sup>*

<sup>1</sup>Royal College of Surgeons in Ireland, Dublin, Ireland, <sup>2</sup>Genealogical Society of Ireland, Dún Laoghaire, Ireland, <sup>3</sup>University of Edinburgh, Edinburgh, United Kingdom, <sup>4</sup>University College Dublin, Dublin, Ireland.

**Aims:** The Irish DNA Atlas is a DNA collection being assembled with the aim of describing the fine-scale population structure in Ireland. Understanding such structure can inform on optimal design of clinical genetic studies as well as the history of the Irish population. We present an overview of, and the preliminary findings from, the study.

**Methods:** We are recruiting individuals with all eight great-grandparents born in Ireland, within 30 kilometres of each other. Participants are asked to complete a detailed birth-brief, which records place and date of birth of three generations of ancestors. DNA is extracted from a saliva sample. We have genotyped using an Illumina OmniExpress dense SNP genotyping platform. We present a number of analyses designed to visualise genetic structure, including: fineStructure, and ADMIXTURE analysis. We compare the Atlas Irish to European populations, and British individuals from the Peoples of the British Isles study.

**Results:** To date we have recruited 211 participants. An analysis of dense genotyping data from 142 participants shows that the Atlas participants present affinity with British individuals in ADMIXTURE analysis, but analysis at higher values of k resolves a component that appears enriched in Ireland, but also found in neighbouring counties. fineStructure analysis identifies geographically aligned genetic substructure within Ireland, and can clearly identify Irish with British 'Planter' ancestry.

**Conclusion:** The data and associated analysis presented here illustrates, for the first time via dense autosomal genotype data, clear resolution of genetic substructure in Ireland, and complements recent similar efforts in the British population.

## P18.036

**Genomic evidence for self-domestication in anatomically modern humans**

*C. Boeckx<sup>1</sup>, C. Theofanopoulou<sup>2</sup>*

<sup>1</sup>ICREA/Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Universitat de Barcelona, Barcelona, Spain.

**Introduction:** The genomic revolution offers previously unavailable bodies of data to test hypotheses concerning the forces that shaped modern cognition. Here we adduce evidence from results in comparative genomics in favor of a role for self-domestication in modern humans.

**Materials and Methods:** We compared lists of genes showing strong signals of positive selection, especially lists of fixed mutations, in anatomically modern human vs. neanderthal (Paabo 2014, Cell), wolf vs. dog (Cagan and

Blass 2016, BMC Evol. Bio.), Large White vs. Tongcheng pig (Li et al. 2014, Animal Genetics), and lab-reared and wild-caught *Drosophila melanogaster* (Stanley and Kulathinal 2016, BMC Evol. Bio.).

Results: We find strong convergence, indeed common targets, in neurogenetic genes, as well as genes implicated in neural crest cells. Particularly in dogs and modern humans, we find genes influencing the velocity of axonal impulse conduction and hormonal changes leading to pro-social dispositions (oxytocin pathways).

Conclusion: The convergence observed suggests that self-domestication played a role in the emergence of cognitive and behavioral human modernity, and also provides partial support for the neural crest cell-based account of the domestication syndrome (Wilkins et al. 2014, Genetics).

#### P18.037

#### Genetic variants influencing epigenetic plasticity

M. Rask-Andersen, S. Enroth, U. Gyllensten, Å. Johansson;

Uppsala university, Uppsala, Sweden.

Introduction: Variance or variation SNPs (vSNPs) are genetic variants associated with phenotypic variance heterogeneity, rather than mean phenotypic value. Such vSNPs could be advantageous for adaptation to new environments. It has been suggested that vSNPs can increase epigenetic plasticity, and thereby even explain part of the missing heritability behind complex diseases. In this study, we have identified vSNPs associated with unequal variances in DNA methylation.

Materials and Methods: DNA methylation levels at over 450,000 CpG sites and genotype data from over 10,000,000 SNPs were analyzed in 729 individuals. The variances for different genotypes were compared using an F test. Results: We could verify that three previously identified vSNPs (rs6936204, rs6936204, and rs3130320) are associated with unequal variance in DNA methylation ( $P < 0.99$ ) with respective vSNP. We also identify 4797 novel vSNPs ( $p < 9.4 \times 10^{-11}$ ) of which a majority (96.7%) are due to additional linked SNPs that alter the mean methylation level of the CpG site. Only a small number of the vSNPs identified ( $N = 132$ ) did not appear to be explained by additionally linked SNPs.

Conclusions: Most vSNPs are not causing epigenetic plasticity, but are rather caused by linked genetic variants that are associated with the phenotypic mean. However, we have identified other potential vSNPs that might influence the variance in DNA methylation, possibly increasing the epigenetic plasticity and thus altering individuals' risk of environmentally induced diseases.

#### P18.038

#### Environmental effects on epigenetic variance in monozygotic twins

E. Carnero-Montoro<sup>1</sup>, A. Visconti<sup>1</sup>, C. Sacco<sup>2</sup>, P. C. Tsai<sup>1</sup>, T. D. Spector<sup>1</sup>, J. T. Bell<sup>1</sup>;

<sup>1</sup>King's College London, London, United Kingdom, <sup>2</sup>Department of Statistical Sciences, University of Bologna, Italy, Bologna, Italy.

It has been proposed that epigenetic modifications play a key role in mediating individuals' phenotypic responses to environmental changes. Recent epigenome-wide association studies (EWAS) have confirmed that environmental exposures, such as smoking, affect DNA methylation levels in human populations. Until now, EWAS have predominantly focused on finding mean differences in methylation levels between exposed and unexposed subjects. We hypothesise that environmental factors can increase epigenetic variability at functionally relevant genomic regions in individuals exposed to environmental stimuli, and that the increased epigenetic variance can trigger phenotypic plasticity in response to environmental exposures.

Monozygotic (MZ) twin studies are extremely valuable to detect environmentally-driven epigenetic changes, as MZ twins are matched for many factors, ruling out the possibility that associated epigenetic modifications are attributed to genetic variation. Here, we used an environment-concordance MZ twin study design to detect CpG sites showing differences in DNA methylation variance between twins exposed and unexposed to smoking, that we define as "environmentally-induced variable methylated sites" (eVMSs). To search for eVMS in a dataset of 490 female Caucasian from the TwinsUK registry, we calculated the difference of DNA methylation within each twin pair as a dispersion measure, and we applied the F-test to detect differences in dispersion values among smoking groups. We validated the genome-wide significant eVMSs using the Brown-Forsythe's test in a subset of 438 unrelated subjects. Furthermore, we explored the relationship between eVMSs and metabolic and healthy ageing traits to understand how risk factors, and the consequent environmental-induced epigenetic variability, may trigger disease-related phenotypic changes.

#### P18.039

#### A genome-wide association study for facial and scalp hair features in admixed Latin Americans

K. Adhikari<sup>1</sup>, T. Fontanil<sup>2</sup>, S. Cal<sup>2</sup>, A. Ruiz-Linares<sup>1</sup>;

<sup>1</sup>University College London, London, United Kingdom, <sup>2</sup>Universidad de Oviedo, Oviedo, Spain.

There is great diversity in primates including humans with regard to hair appearance, such as its distribution, shape and color. Hair plays a range of important functions, including thermal regulation, camouflage, sensory and social signaling, and the evolution of hair has been proposed to be influenced by both natural and sexual selection. Patterns of hair appearance are highly heritable, showing distinct differences across continents. Association studies, mostly in European and East Asian samples, have found loci corresponding to scalp hair color, shape and male pattern baldness.

In a genome-wide association study of admixed Latin Americans from five different countries, we identified eighteen genome-wide significant associations for features of scalp hair (shape, color, graying, balding) and facial hair (beard thickness, mono-brow, eyebrow thickness), including ten novel associations. These contain the first reported loci for hair graying, mono-brow, and eyebrow and beard thickness. A newly identified locus influencing hair shape includes a Q30R substitution in the Protease Serine S1 family member 53 (PRSS53). This enzyme appears to be highly expressed in the hair follicle, especially the inner root sheath, and that the Q30R substitution affects enzyme processing and secretion. The identified regions can have implications for both forensics and the cosmetics industry. Consistent with proposals regarding the evolution of human hair, the genomic regions associated with hair features are enriched for signals of selection.

#### P18.040

#### High prevalence of monogenic familial hypercholesterolemia in a population based study needs deep genetic dissection

m. banikazemi<sup>1,2</sup>, M. Dameshpour<sup>1</sup>, S. Masjoodi<sup>1</sup>, S. Zoroufchin<sup>1</sup>, B. SedaghatiKhayat<sup>1</sup>, K. Guity<sup>1</sup>, F. Azizi<sup>3</sup>;

<sup>1</sup>Cellular and Molecular Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>New York Medical College, Valhalla, NY, United States, <sup>3</sup>Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Introduction: Cardiovascular disorders are the most important health issues in most countries. Dyslipidemia are in high correlation with coronary cardiovascular disorders. In current study we aim to mark and determine prevalence of inherited forms of hypercholesterolemia in Tehran lipid and glucose study (TLGS) and select families for further studies of genetic pattern of this disorder.

Methods: Anthropometrics and biochemical measurements from 16,785 individuals older than 18 years enrolled in each five phases of TLGS were analyzed using Simon Broome criteria. Also individual with triglyceride more than 200 mg/dl were excluded as confounder. Each selected individual's full family pattern was drawn by Progeny software and reviewed in detail.

Results Overall prevalence of dyslipidemia in TLGS was determined to be around 13.9 % in subjects older than 18 years of age (58.2% females, 41.8% males). After reviewing all biochemical and demographic factors related with dyslipidemia, diabetes mellitus type II and obesity were ascertained as major contributing risk factors especially after 40 years old. The results also demonstrated increase in lipid lowering treatment rate along the study phases reaching around 40% during phase five of TLGS. Isolated familial hypercholesterolemia was seen only in 91 individuals (73 Families) with estimated prevalence of 0.005.

Conclusion:

Prevalence of dyslipidemia (elevated TC, LDL and TG) was relatively high in TLGS study cohort (13.86%) and in older age was associated with other comorbidity factors such as diabetes and obesity. However, inherited forms of isolated familial hypercholesterolemia prevalence were comparable to reported published data from other countries and communities.

**P18.041****Cancer surveillance program in young adult patients with Fanconi Anemia (FA)**

*E. Carrasco<sup>1</sup>, N. Gadea<sup>2</sup>, I. Esteban<sup>1</sup>, S. Torres<sup>1</sup>, C. Diaz<sup>3</sup>, D. Valcarcel<sup>4</sup>, C. Centeno<sup>5</sup>, M. Munill<sup>6</sup>, J. Lorente<sup>7</sup>, M. Pujo<sup>8</sup>, J. Surrallés<sup>9</sup>, J. Balmaña<sup>12</sup>;*

<sup>1</sup>Familial Cancer Program, Vall D'Hebron Institute of Oncology, Barcelona, Spain,

<sup>2</sup>Medical Oncology Department, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>3</sup>Pediatric Hematology and Oncology Department, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>4</sup>Hematology Department, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>5</sup>Gynecology Department, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>6</sup>Department of Oral and Maxillofacial Surgery, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>7</sup>Otorhinolaryngology Department, University Hospital Vall d'Hebron, Barcelona, Spain,

<sup>8</sup>Genome Instability and DNA Repair Group, Department of Genetics and Repair Group, Department of Genetics and Microbiology, Universitat Autònoma de

Barcelona (UAB), Barcelona Spain and Centre for Biomedical Network Research on Rare

Diseases (CIBERER), Barcelona, Spain.

**Introduction:** Young adult patients with FA face a high risk of solid malignancies. Efforts have been developed to promote follow-up programs for early cancer detection (Spanish guidelines from the *Red Nacional de Anemia de Fanconi*, 2012). Here we aim to describe our 6-year experience and the strategies developed to enhance compliance and reduce patient's cancer risk.

**Materials and Methods:** From 26 FA patients, 58% are male, median age 21 (17 -34y), 11 are current or former smokers. Patients undergo a full anamnesis and receive health education in the high risk clinics and are recommended a surveillance program that includes hematological, head and neck, oral cavity, and gynecologic (females) follow up every 6 months.

**Results:** All women have had HPV vaccination, and one premalignant cervical lesion was detected (CIN1). In two patients (8%) bone marrow aspirate yielded a diagnosis of myelodysplastic syndrome. Seven patients (27%) were diagnosed with a malignant tumor. In a biallelic *BRCA2* patient with a prior breast cancer, a secondary *in situ* breast cancer was detected in her prophylactic contralateral mastectomy. The other six patients had a tumor in the head and neck area (HNSCC). At maxilofacial follow-up, two *in situ* SCC in the oral cavity were diagnosed.

**Conclusion:** HNSCC are the most frequent neoplasm in young adults with FA. Intensive surveillance and preventive strategies are needed. Avoiding tobacco and counseling about healthy lifestyle habits are mandatory to minimize their risk of solid tumors. Increasing self-awareness of oral changes to seek medical evaluation are warranted for early detection.

**P18.042****GCAT|Genomes for Life: A prospective cohort study of the genomes of Catalonia**

*M. Obón-Santacana<sup>1,2</sup>, M. Vilardell<sup>2</sup>, A. Carreras<sup>2</sup>, X. Duran<sup>2</sup>, G. Valeta<sup>3</sup>, L. Puig<sup>3</sup>, M. Perucho<sup>4,5</sup>, L. Sumoy<sup>6</sup>, E. Duell<sup>7</sup>, V. Moreno<sup>1</sup>, R. de Cid<sup>2</sup>;*

<sup>1</sup>Cancer Prevention and Control Program, Catalan Institute of Oncology, ICO-IDIBELL, Barcelona, Spain, <sup>2</sup>Genomic Diseases-GCAT lab Group, Institute of Predictive and Personalized Medicine of Cancer (IMPPC)- Germans Trias Health Research Institute (IGTP), Can Ruti Campus Badalona, Spain, Badalona, Spain, <sup>3</sup>Blood and Tissue Bank (BST), Barcelona, Spain, Barcelona, Spain, <sup>4</sup>Cancer Genetics and Epigenetics Program, Institute of Predictive and Personalized Medicine of Cancer (IMPPC)- Germans Trias Health Research Institute (IGTP), Can Ruti Campus Badalona, Spain, Badalona, Spain, <sup>5</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, <sup>6</sup>Genomics and Bioinformatics Group, Institute for Predictive and Personalized Medicine of Cancer (IMPPC), Can Ruti Campus, Badalona, Spain, <sup>7</sup>Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain.

**Introduction:** The prevalence of chronic diseases is increasing; thus large prospective cohorts studies are needed to increment the genetic and epidemiological knowledge of these conditions.

**Materials&Methods:** The Genomes for Life project develops a multi-purpose prospective cohort study that aims to identify genomic, epigenomic, environmental and lifestyle factors, as well as their interactions involved in the development of cancer and other diseases (i.e. cardiovascular, respiratory, metabolic). At recruitment, participants reported information on lifestyle/dietary habits, drug use, diseases diagnostics, family history of diseases, and medical interventions using questionnaires. Information on anthropometric measures, blood pressure, rate pulse, and blood sample were also collected for each participant. Started in the mid-2014 (still ongoing), the population is being recruited through the Blood and Tissue Bank network from Public Healthcare users, aged 40-65 years, and with current residence in the Catalan region. Participants will regularly update their exposure data and will be followed over time for the occurrence of events and mortality by linkage to personal clinical records from the Catalan Public Healthcare.

**Results:** In February-2016, the GCAT included 9.500 volunteers. The present analysis is based on a subset of 8.910 observations, of which 59% were women. 72% of the participants reported having a good self-perceived health, and according to the WHO classification, 54% were overweight. The

most common self-reported diseases were hypercholesterolemia, allergies, and hypertension (18%, 17%, and 15% respectively), and 4% of participants had diabetes. Currently 3.500 blood samples have been genotyped for further analyses.

**Conclusions:** The GCAT project represents a platform for implementation of the genomic medicine on chronic disorders.

**P18.043****Population genetic carrier screening tests : The Israeli national program experience**

*A. Singer<sup>1</sup>, I. Grotto<sup>2</sup>, P. Stafler<sup>3</sup>, C. Vinkler<sup>4</sup>;*

<sup>1</sup>Community Genetics, Public Health Services, Ministry of Health, Jerusalem, Israel,

<sup>2</sup>Public Health Services, Ministry of Health, Jerusalem, Israel, <sup>3</sup>Cystic Fibrosis Center, Schneider Children's Medical Center of Israel, Petach Tikva, Israel, <sup>4</sup>Inst Medical Genetic Wolfson Medical Center, Holon, Israel.

The Israeli population is combined of various ethnic groups which are quite conserved. These groups are Jewish (Ashkenazi and non-Ashkenazi) and arabs (Muslim, Christian, Druze). High frequency of genetic diseases is unique to the Israeli population as a result of its religious isolated small communities, and high rate of consanguinity. Founder mutations are known to be present in these communities and were identified over the years (e.g. Tay Sachs).

With the possibility to perform a molecular diagnosis of many genetic diseases, many disorders became candidates for screening. The Israeli Society of Medical Geneticists (ISMG) made recommendations to expand the carrier screening program and include all of the severe genetic diseases in which the carrier frequency has 1:60. The Israeli MOH accepted this recommendations and today 18 tests are offered free of charge to the general population. This program offers carrier screening for cystic fibrosis, fragile X, and spinal muscular atrophy for the general population and additional tests for severe diseases with high carrier frequency to specific ethnic groups according to founder mutation.

**Methods:** Tests are offered by the primary physician as part of preconception recommendations. Pretest information are given by trained professionals. Carriers are referred to genetic counseling for discussion of prenatal tests options.

**Results:** More than 400,000 tests were done during 2013-2014 for the above 18 traits. Of these 117,291 were CF carrier screening tests. The CF registry proves the impact of this program showing a marked reduction in CF birth rates with a shift towards milder mutations.

**P18.044****A generalized multilevel adjusted latent class mixed modeling approach for genome-wide association study of blood pressure in a longitudinal pedigree framework**

*Ö. Karadag, S. Aktaş;*

*Hacettepe University Department of Statistics, Ankara, Turkey.*

**Introduction:** In order to find out the gene regions causing the disease of interest, association studies require a whole genome sequenced framework. Complex designs such as longitudinal measurements are useful tools to understand the behavior of the disease phenotype. In addition, family-based designs provide evidence about the heredity of the disease. In the presence of multiple correlated data, regarding the dependency between the observations may cause assumption violations. Hence, an advanced association model related the correlation structures should be used for testing the whole genome association in a longitudinal pedigree framework.

In this study we propose a generalized multilevel adjusted latent class mixed model for testing the association through the whole genome accounting both serial and the familial correlations by using a latent class structure for the heterogeneous longitudinal phenotype distribution.

**Materials and Methods:** Genetic Analysis Workshop data was used for the implementation of the proposed model. The longitudinal blood pressure trajectories are homogenized by using latent classes. Because of the computational restrictions, rare variants with  $MAF < 0.05$  are excluded from the analysis. Familial aggregation was integrated into the model by calculating the kinship matrix.

**Results:** Odd numbered chromosomes were analyzed and the genome wide association results were displayed by using Manhattan plots. Parameter and the heritability estimates were calculated for remarkable gene regions.

**Conclusions:** The proposed model enables to analyze a considerable amount of SNPs by accounting both serial and familial correlations in the parameter estimations and according to results, provides a better fit rather than the classical models.

## P18.045

**Choosing the right genotyping array: more than just coverage**

L. Broer<sup>1</sup>, E. Clemens<sup>2,3</sup>, O. Zolk<sup>4</sup>, A. Verkerk<sup>1</sup>, A. am Zehnhoff-Dinnens<sup>5</sup>, J. Hottenga<sup>6</sup>, C. Lanvers-Kaminsky<sup>7</sup>, T. Langer<sup>8</sup>, M. M. van den Heuvel-Eibink<sup>2,3</sup>, A. G. Uitterlinden<sup>1</sup>;

<sup>1</sup>Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands,

<sup>2</sup>Department of Pediatric Oncology, Erasmus Medical Center, Rotterdam, Netherlands,

<sup>3</sup>Department of Pediatric Oncology, Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands,

<sup>4</sup>Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Ulm, Germany, <sup>5</sup>Clinic of Phoniatrics and Pedaadiology, University Hospital Münster, Münster, Germany, <sup>6</sup>Department of Biological Psychology, VU University Amsterdam, Amsterdam, Netherlands, <sup>7</sup>University Children's Hospital Muenster, Department of Pediatric Hematology & Oncology, University Hospital Muenster, Muenster, Germany, <sup>8</sup>University Hospital for Children and Adolescents Lübeck, Department of Pediatric Oncology & Hematology, Lübeck, Germany.

Since the dawn of GWAS the number of genotyping arrays has exploded, which makes the choice of array challenging. We compared all currently available arrays on several array characteristics (coverage and imputation efficiency) and focused on several SNP categories such as pharmacogenetics, actionable genes and mtDNA.

SNP-manifest-files for all arrays were obtained from Illumina and Affymetrix. Calculations of coverage were based on the 1kG reference panel, which was divided in its separate ancestries. Imputations using HapMap samples were performed to 1kG. For pharmacogenetics 388 genes and for "actionable genes" 56 genes proposed by ACMG were considered. For mtDNA 71 mt-loci were evaluated.

Twenty-three arrays were included in the comparison. The coverage increased with the size (number of variants) of the array. Imputation quality also increased with the size of the array, indicating that coverage is a good predictor of imputation quality. For both pharmacogenetics and actionable genes a similar trend was observed. However, for pharmacogenetics the DrugDev array from Illumina had over double the number of variants in these genes compared to other arrays of similar size ( $P<0.0001$ ). Similarly, for actionable genes the Axiom\_UKB had 7 times more variants than expected ( $P<0.0001$ ). No trend was observed for mtDNA, however 3 of the arrays had no mtDNA content, while the global array had a much larger mtDNA content than average ( $P=0.001$ ).

In conclusion, choosing the right genotyping array will depend on the questions to be answered. However, comparing the different arrays using objective criteria can help make this decision more manageable.

## P18.046

**Identification of a novel locus associated with skin color in African-admixed populations**

N. Hernandez-Pacheco<sup>1,2</sup>, E. G. Burchard<sup>3,4</sup>, S. Alonso<sup>5</sup>, C. Eng<sup>3</sup>, S. Hunstman<sup>3</sup>, D. Hu<sup>3</sup>, C. Flores<sup>1,2,6</sup>, M. Pino-Yanes<sup>1,6</sup>;

<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain,

<sup>2</sup>Applied Genomics Group (G2A), Section of Genetics, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San Cristobal de La Laguna, Tenerife, Spain, <sup>3</sup>Department of Medicine, University of California, San Francisco, CA, United States, <sup>4</sup>Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA, United States, <sup>5</sup>Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain, <sup>6</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

**Introduction:** The identification of genetic variants involved in determining skin color has anthropological and forensic importance and could help to identify genetic factors involved in the susceptibility to skin cancer. Here we aimed to uncover genes associated with skin pigmentation in African-admixed populations.

**Materials and Methods:** We performed a genome-wide association study (GWAS) of melanin levels in 285 Puerto Ricans genotyped with the Axiom LAT1 array (Affymetrix). Imputation of genetic variants was performed using The Haplotype Reference Consortium data by means of the Michigan Imputation Server. A total of 11 million variants with minor allele frequency  $\geq 1\%$  and  $R^2 \geq 0.3$  were analyzed for association using EPACTS. Variants with  $p \leq 1 \times 10^{-5}$  were followed up for replication in 373 African Americans genotyped with the same array.

**Results:** A total of 82 variants associated with melanin levels in Puerto Ricans at  $p \leq 1 \times 10^{-5}$ . Seventy-seven were accurately imputed in African Americans, 14 of which showed evidence of replication. A meta-analysis of the two samples identified nine genome-wide significant hits: seven of them located within two genes already known to be associated with skin color (*SLC24A5* [ $\min p\text{-value} = 2.6 \times 10^{-14}$ ] and *SLC45A2* [ $\min p\text{-value} = 9.7 \times 10^{-10}$ ]), and two variants located in an intergenic region between *BEND7* and *PRPF18* ( $\min p\text{-value} = 4.6 \times 10^{-9}$ ), a locus associated with skin color for the first time.

**Conclusions:** Our GWAS in African-admixed populations confirmed the im-

portance of two loci identified by previous studies and revealed the contribution of a new locus in skin pigmentation.

## P18.047

**Exploring the ancestry of modern-day Tuscans**

S. Anelli<sup>1,2</sup>, C. Di Gaetano<sup>1</sup>, S. Guarnera<sup>2,1</sup>, G. Matullo<sup>1,2</sup>, A. Piazza<sup>1,2</sup>;

<sup>1</sup>University of Turin, Turin, Italy, <sup>2</sup>HuGeF, Turin, Italy.

**Background.** Etruscan civilization flourished in central Italy from the eighth century BC to the first century AD. A theory of its origin was reported by Herodotus (484-425 BC), who guessed Etruscans came from Asia Minor. In Lemnos, an Aegean island, a stele with inscriptions similar to Etruscan language was found in 1885. Several genetic studies with conflicting pieces of evidence have been accumulated. We analysed the genetic relationships of modern Tuscans with other Aegean peoples by comparing 668,506 DNA SNPs in 122 unrelated individuals living since many generations where the historical Etruscans were settled (i.e. Murlo, Viterbo, Ferrara) and Lemnos Island ( $n=16$ ).

**Results.** Several identity-by-state (IBS) analyses pointed to a stratification of different genetic contributions to the Tuscan modern genome. Ancestry proportions computed using the ADMIXTURE code showed a predominance of Southern European and Middle Eastern components ( $38.0\% \pm 2.8\%$  and  $40.3\% \pm 3.8\%$ , respectively). Principal component analysis highlighted the genetic relationship of Tuscans to Southern Europe in addition to that with Greek and Aegean individuals.

**Conclusion.** The high resolution analysis of the modern Tuscan genome exhibits a genetic signature broadly consistent with strata from Lemnos and Greece on a genetic background contributed by Middle Eastern and neighbouring Southern Italian individuals. Such results, to be corroborated by testing more and geographically better distributed individuals, are leading to a radical change of our paradigm of population genetic studies by rephrasing the often abused concept of population origin.

**Grant references:** HuGeF, a Research Foundation mainly granted by Compagnia di San Paolo, Torino, Italy supported this study.

## P18.048

**Highly differentiated variants in Africa: Genetic differentiation vs. migration**

L. R. Botigué, B. M. Henn;

Stony Brook University, Stony Brook, NY, United States.

**Introduction:** An individual's genome is composed primarily of common variants, which are not uniformly distributed across all human populations. This uneven distribution is especially significant in Africa, characterized by a complex genetic structure, and where dramatic variation in allele frequencies is found across populations.

**Materials and Methods:** We analyze low coverage whole genomes from 3,400 Africans sampled from a dozen populations throughout the continent in order to quantify highly differentiated variants (HDVs). HDVs are variants common in one population but rare in another. We perform an enrichment analysis to test whether HDVs are enriched for specific biological functions, and we use population genetic theory to analytically predict the number of expected HDVs.

**Results:** We see little differentiation among Bantu-speaking populations, emphasizing the strong genetic footprint that the Bantu expansion left in sub-Saharan Africa. We find that HDVs are more affected by genetic drift than by time since divergence between populations, and we detect an exponential relationship between the number of HDVs and Fst. We identify some biological functions that are enriched in HDVs, though predictions of the number of HDVs do not deviate from observed HDVs.

**Conclusions:** Overall, we conclude that the number of HDVs found between populations is mainly due to demography rather than natural selection even though a large fraction of HDVs are likely to be functional.

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## P18.049

**Genome-wide association studies of Immunoglobulin G glycans**

L. Klarić<sup>1,2,3</sup>, M. Mangino<sup>4</sup>, T. Esko<sup>5,6,7</sup>, P. Salo<sup>8</sup>, Y. A. Tsepilov<sup>9</sup>, F. Vuckovic<sup>2</sup>, I. Ugrina<sup>10</sup>,

R. Plomp<sup>11</sup>, M. Pucic-Bakovic<sup>2</sup>, J. E. Huffmann<sup>1</sup>, M. Wuhrer<sup>11</sup>, M. Dunlop<sup>12</sup>, M. Perola<sup>8</sup>, K. Fischer<sup>5</sup>, H. Campbell<sup>9</sup>, O. Polasek<sup>13</sup>, I. Rudan<sup>3</sup>, J. Wilson<sup>3</sup>, Y. S. Aulchenko<sup>9,14</sup>, T. Spector<sup>4</sup>, C. Hayward<sup>1</sup>, G. Lauc<sup>2,10</sup>,

<sup>1</sup>Medical Research Council, Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>2</sup>Genos Glycoscience

Research Laboratory, Zagreb, Croatia, <sup>3</sup>Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom, <sup>4</sup>Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom, <sup>5</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>6</sup>Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, MA, United States, <sup>7</sup>Division of Endocrinology, Boston Children's Hospital, Cambridge, MA, United States, <sup>8</sup>Genomics and Biomarkers Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland, <sup>9</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>10</sup>Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia, <sup>11</sup>Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands, <sup>12</sup>Colon Cancer Genetics Group, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>13</sup>Department of Public Health, Faculty of Medicine, University of Split, Split, Croatia, <sup>14</sup>PolyOmica, Groningen, Netherlands.

Glycans are complex carbohydrates attached to the surface of the protein. With glycosylation being amongst the most abundant post-translational modification, glycans have important role in many physiological processes. Almost forty years ago they were first shown to be changed in patients with rheumatoid arthritis, and ever since the number of diseases associated with glycosylation has been growing.

We have performed genome-wide association studies of 77 immunoglobulin G (IgG) glycosylation traits on 8000 samples imputed to HapMap2 reference panel originating from four different European cohorts with replication in four additional European cohorts (n = 4500). Conditional and joint analyses have been performed using Genome-wide Complex Trait analysis software. Data-Driven Expression-Prioritized Integration for Complex Traits has been used to prioritise genes within associated loci.

More than a hundred novel hits mapping to more than thirty loci have been discovered, many of which were previously shown to be associated with various autoimmune diseases, implicating potential pleiotropy of IgG glycosylation and diseases such as Inflammatory Bowel Disease and Systemic Lupus Erythematosus. Gene sets and pathways enriched for association with glycosylation have been assessed, providing additional insights in the mechanisms of protein glycosylation.

With this study we expanded the network of genes known to be involved in glycosylation of immunoglobulin G providing us with further insights how these molecules could be involved in complex human diseases.

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#### P18.050

#### Association between INADL polymorphisms and ischemic stroke functional outcome through genome wide association meta-analysis. GOS Project

*M. Mola-Caminal<sup>1,2</sup>, C. Soriano-Tárraga<sup>1</sup>, E. Giralt-Steinhauer<sup>1</sup>, C. Carrera<sup>3</sup>, R. Rabionet<sup>4</sup>, I. Fernández-Cadenas<sup>5</sup>, C. Vives-Bauzá<sup>6</sup>, X. Estivill<sup>7</sup>, J. Roquer<sup>8</sup>, J. Jiménez-Conde<sup>9</sup>, <sup>1</sup>Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain, <sup>2</sup>University of Barcelona (UB), Barcelona, Spain, <sup>3</sup>Vall d'Hebron Research Institute (VHIR), Barcelona, Spain, <sup>4</sup>Centre for Genomic Regulation (CRG), Barcelona, Spain, <sup>5</sup>Fundació Docència i Recerca Mútua Terrassa, Terrassa, Spain, <sup>6</sup>Fundació d'Investigació Sanitària de les Illes Balears (FISIB), Palma de Mallorca, Spain.*

Cerebrovascular disease is one of the leading causes of disability in adults. Variability in functional outcome after ischemic stroke can be influenced by many factors. Irrespective of clinical factors such as age, stroke subtype, vascular stenosis, location of the injury and size of the affected area, inter-individual variation in neuronal recovery is considerable. A number of metabolic pathways are involved in the cerebral ischemic damage response and their activity may be modulated by variation in the genes that encode their various components. Our aim was to identify genetic variants and genes influencing the recovery process.

We conducted a meta-analysis of four different genome-wide association studies. We included 1193 anterior territorial ischemic stroke Spanish cases with modified Rankin functional Scale measures at 3 months after stroke. Peripheral blood cell DNA was genotyped using the Illumina HumanCore-Exome-24-BeadChip. Association analyses with outcome at 3 months after ischemic stroke were performed using SNPTEST and meta-analyzed with METAL. Data was adjusted for clinical severity at discharge measured by National Institute of Health Stroke Scale, hypertension, age, gender and principal components. Imputation was done using IMPUTE2.

We discovered three genetic variants associated with 3 months outcome after ischemic stroke located in the INADL gene (<5.3E-08).

We have identified a potential loci suggestive of being relevant for functional outcome and the recovery process after stroke. Follow-up studies are warranted to validate these findings in other populations and to establish whether INADL can be a novel therapeutic target for the rehabilitation strategies. Funding: Fundació la Marató de TV3

#### P18.051

#### Improving the power of longevity GWAS by using disease candidates, also investigating pleiotropic effects on longevity via multiple diseases

*A. F. McDaid<sup>1</sup>, J. Murabito<sup>2</sup>, Z. Katalik<sup>3</sup>,*

*<sup>1</sup>Institute of Social and Preventive Medicine (IUMSP), Centre Hospitalier Universitaire Vaudois (CHUV), 1010 Lausanne, Switzerland, <sup>2</sup>Boston University School of Medicine, Boston, MA, United States.*

GWAS studies for longevity have found only two robustly replicating SNPs in and near APOE and FOXO3. Recent literature has improved power by looking at subsets of SNPs associated with specific diseases (Fortney et al, PLOS Genetics, Dec 2015). We improve on previous work in various aspects: (i) combined together the two largest longevity GWAS meta-analysis studies ; (ii) used a large panel (190 studies) of genome-wide association summary statistics and applied Mendelian Randomization to compute a causal estimate of various traits/diseases on longevity. For example, using the top 15 (independent) LDL-increasing SNPs we estimate that high LDL levels reduce the chance to live longer than 90 years (-0.24 log odds / LDL SD unit, with p<3e-15).

For each SNP we built priors for longevity effect by combining the SNP's effect on different diseases and the causal effect of these diseases on longevity. This way pleiotropic SNPs with even with small impact on individual traits can gain solid prior strength. We then calculated the Bayes factor for each marker and found that several loci gained genome-wide significance thanks to the informative prior. Furthermore, our approach allowed for dissecting longevity heritability to genetic factors that act through the examined diseases and those that do not. This analysis revealed that the genetic determinants of longevity strongly overlap with those that render us susceptible to diseases.

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#### P18.052

#### Genetic determinants of pigmentation in admixed Latin Americans

*J. Mendoza-Revilla<sup>1</sup>, K. Adhikari<sup>1</sup>, V. Acuña-Alonso<sup>1</sup>, C. Gallo<sup>2</sup>, G. Poletti<sup>2</sup>, M. Bortolini<sup>3</sup>, F. Rothhammer<sup>4</sup>, G. Bedoya<sup>5</sup>, S. Canizales-Quinteros<sup>6</sup>, R. Gonzalez-José<sup>7</sup>, D. Balding<sup>8</sup>, A. Ruiz-Linares<sup>1</sup>, CANDELA Consortium;*

*<sup>1</sup>University College London, London, United Kingdom, <sup>2</sup>Universidad Peruana Cayetano Heredia, Lima, Peru, <sup>3</sup>Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil,*

*<sup>4</sup>Universidad de Tarapacá, Arica, Chile, <sup>5</sup>Universidad de Antioquia, Medellín, Colombia,*

*<sup>6</sup>Universidad Nacional Autónoma de México, Mexico DF, Mexico, <sup>7</sup>Centro Nacional Patagónico, CONICET, Puerto Madryn, Argentina, <sup>8</sup>University of Melbourne, Melbourne, Australia.*

Human pigmentation is a striking aspect of human variability, showing remarkable variation between individuals and populations. In recent years, association studies have identified a number of genes affecting common variation in pigmentation, highlighting the complex genetic architecture of this phenotype. The majority of these studies have been carried out in European-related populations. To better understand the genetic architecture of human pigmentation variation, and its evolution, analyses in a wider range of populations are needed.

We conducted a genome-wide association scan in over 6,000 Latin Americans for three pigmentation traits: skin, eye and hair color. Association testing used multivariate linear regression with an additive genetic model adjusting for: age, sex, and the first five principal components obtained from the SNP data.

Our analyses revealed eleven independent genome-wide significant signals of association with SNPs at 8 different genomic regions including a novel candidate region associated with skin pigmentation. We found that the associated SNP had a higher derived allele frequency in East Asians compared to Europeans and that it showed strong signals of positive selection in East Asians, consistent with the independent evolution of light skin in Eastern Eurasians. Ongoing functional analyses in skin melanocytes should help explain the biological basis of the association observed.

In conclusion, we identified a novel region impacting on human skin pigmentation, providing further insights into the genetics and evolution of this complex trait.

## P18.053

**Statistical tests for Hardy-Weinberg equilibrium for bi-allelic genetic markers at the X chromosome***J. Graffelman<sup>1</sup>, B. S. Weir<sup>2</sup>;*<sup>1</sup>Universitat Politècnica de Catalunya, Barcelona, Spain, <sup>2</sup>University of Washington, Seattle, WA, United States.

**Introduction:** The X chromosome complicates much of genetic data analysis due to the fact that males carry only one copy. Even the most basic analysis, a test for Hardy-Weinberg equilibrium (HWE), is complicated by the hemizygous males. Hitherto, the standard approach for testing X-chromosomal markers has been to ignore the males. In this contribution we argue that males are relevant, because a difference in male and female allele frequency indicates that equilibrium does not hold.

**Materials and Methods:** We develop chi-square, likelihood-ratio, exact and permutation tests for bi-allelic markers at the X chromosome, assuming a multinomial distribution for the data considering all five genotype counts. We study the Type I error rate of the proposed tests, and compare this approach with the standard tests that exclude males. Empirical data from the GENEVA project on venous thromboembolism is used to assess the practical usefulness of the new methods.

**Results:** Our X chromosomal exact test (with mid p-value) is most promising because its rejection rate is most close to the nominal level under the HWE assumption. It shows better convergence to the nominal significance rate in comparison with the standard exact test which excludes males. The analysis of the GENEVA database reveals HWE test results can experience large changes in p-value when the males are included.

**Conclusions:** We have designed very basic statistical tools for the analysis of X-chromosomal genotype data. Our methods are omnibus procedures that verify Hardy-Weinberg proportions and equality of male and female allele frequencies in a single test.

## P18.054

**GWAS of rejection and mortality in heart transplant patients***J. van Setten<sup>1</sup>, Y. R. Li<sup>2</sup>, N. de Jonge<sup>1</sup>, M. V. Holmes<sup>2</sup>, C. C. Baan<sup>3</sup>, O. C. Manintveld<sup>2</sup>, A. M. A. Peeters<sup>3</sup>, F. Dominguez<sup>4</sup>, P. Garcia-Pavia<sup>4</sup>, K. K. Khush<sup>5</sup>, J. W. Rossano<sup>2</sup>, R. A. de Weger<sup>4</sup>, B. Keating<sup>2</sup>, F. W. Asselbergs<sup>1</sup>;*<sup>1</sup>University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>University of Pennsylvania, Philadelphia, PA, United States, <sup>3</sup>Erasmus Medical Center, Rotterdam, Netherlands,<sup>4</sup>Puerta de Hierro University Hospital, Madrid, Spain, <sup>5</sup>Stanford University, Stanford, CA, United States.

**Introduction:** Currently, donor/recipient (D-R) matching is suboptimal. Besides HLA, also other genetic factors play a role in graft rejection. We aim to identify genetic variation associated with rejection and mortality after heart transplantation.

**Methods:** iGeneTRAiN is a large-scale international consortium, which consist of over 11,500 solid organ D-R pairs, including over 1,000 heart transplant D-R pairs. We have conducted genome-wide association studies for rejection and for mortality one year after transplantation, testing over 13 million variants in 605 recipients of European descent. We aim to perform association testing in a larger sample of heart transplant recipients and also cross-organ including lung, liver, and kidney transplants, maximizing statistical power to identify novel genetic variants. Importantly, we have high-resolution data of the MHC region, including 4-digit HLA types.

**Results:** We did not identify novel loci for rejection and mortality after heart transplantation. Fourteen loci reached a suggestive P-value ( $P < 1 \times 10^{-6}$ ).

**Conclusions:** We aim to identify variants associated with mortality and rejection after heart transplantation. While our current sample of 605 individuals did not yield novel loci, we will increase the sample size to over 1,000 patients from multiple ancestries. Possible sources of genetic variation underpinning rejection are homozygous deletion copy number variants spanning whole gene or exon regions and LoF variants ablating two copies of a given gene, resulting in incompatibility across the proteomes of donor and recipient. We ultimately aim to translate genetic data into clinical applications such as more optimal genomic compatibility matching of D-R pairs and immune suppression therapy dosing.

## P18.055

**Alpha globin gene mutations and rare variants in Antalya Province, Turkey***I. Keser<sup>1</sup>, Y. Arıkan<sup>1</sup>, T. Karaman<sup>1</sup>, T. Bilgen<sup>2</sup>, D. Canatan<sup>3</sup>, A. Kupesiz<sup>4</sup>;*<sup>1</sup>Department of Medical Biology and Genetics, Medical Faculty, Antalya, Turkey,<sup>2</sup>Department of NABILITEM, Tekirdag, Turkey, <sup>3</sup>Mediterranean Blood Diseases Foundation, Antalya, Turkey, <sup>4</sup>Department of Pediatric Hematology, Medical Faculty, Antalya, Turkey.

Haemoglobinopathies constitute entities that are generated by either an

abnormal haemoglobin or thalassaemias. Of these, Alpha thalassemia is a blood disorder which is characterized by absence or diminished production of alpha globin protein. Mostly deletions affects production of related protein differently. In this study, we performed investigate alpha globin gene mutations among 75 cases with alpha thalassemia in Antalya region, following reverse dot-blot hybridization and DNA sequencing for HBA1 ve HBA2 genes. We found 25 mutations out of 75 cases with alpha thalassemia trait phenotype and revealed that 2 out of 25 mutations were point mutation when 23 cases were found to be in deletional type mutation. Mutation negative cases on strip analyses in 2 different cases, HbG-Waimanalo and Hb- Fontainebleau were firstly discovered as two rare variants by DNA sequencing in Antalya. Deletional mutations were: 10 cases with MED, 5 cases with 20.5 kb, 8 cases with 3.7 kb gene deletion heterozygously and 2 cases with same deletion as homozygously. According to our results MED deletion is the most prevalent alpha thalassemia mutation in Antalya region. But, lastly, DNA sequencing method should be considered in cases with clinical background when there is no mutation on strip assay. Also, found the rare variants' origins should be investigated about geographical distribution. As a result, for identifying alpha-hemoglobinopathies and to give reliable genetic counseling, resolution power of the molecular tests are need to be well-evaluated.

## P18.056

**Trans-ethnic meta-analysis of Hirschsprung disease***C. S. Tang<sup>1</sup>, H. Gui<sup>1</sup>, S. S. Cherny<sup>1</sup>, P. C. Sham<sup>1</sup>, P. K. Tam<sup>1</sup>, J. H. Kim<sup>2</sup>, The International Hirschsprung Disease Consortium, M. M. Garcia-Barceló<sup>1</sup>;*<sup>1</sup>The University of Hong Kong, Hong Kong, Hong Kong, <sup>2</sup>Sogang University, Seoul, Korea, Republic of.

Hirschsprung disease (HSCR), also known as aganglionic megacolon, is the most common cause of neonatal intestinal obstruction, occurring in 1 out of 5000 newborns. It is a congenital disorder characterized by an absence of ganglion cells in the nerve plexuses of the lower intestinal tract. Despite its high heritability, genetic factors predisposing to HSCR remain elusive. So far association of three disease-susceptibility loci—*RET*, *SEMA3* and *NRG1*—were detected from genome-wide association analyses (GWASs) in Europeans and Asians; however, much of the heritability remains unexplained. Here we present the largest trans-ethnic meta-analysis of HSCR (507 cases and 1191 controls), summarizing association results of all three published GWASs on the disease. By leveraging the trans-ethnic differences in linkage disequilibrium, we fine-mapped the three known loci and narrowed down the putatively causal variants to 99% credible sets. We observed no heterogeneity in effect for *RET* and *NRG1* across European and Asian ancestries but detected European-specific association of a low-frequency variant, rs80227144, in *SEMA3* locus ( $OR=5.2$ ,  $P=4.7 \times 10^{-10}$  in European GWAS). Conditional analysis on the lead SNPs further revealed a secondary association signal, corresponding to an Asian-specific, low-frequency missense variant encoding *RET* p.Asp489Asn (rs9282834,  $OR=20.3$ , conditional  $P=4.1 \times 10^{-14}$  in Asian-specific meta-analysis). Effect of *RET* p.Asp489Asn appeared to be non-additive, with an increased risk of HSCR under a compound heterozygous state with the *RET* intron 1 enhancer. Overall, our study provides novel insights into the genetic architecture of HSCR and has profound implications for the future study design.

## P18.057

**Genetic network as post-GWAS analysis approach for correlated complex phenotypes: example on infectious diseases***A. Gelemanović<sup>1</sup>, I. Patarčić<sup>1</sup>, A. Relja<sup>1</sup>, C. Hayward<sup>2</sup>, I. Rudan<sup>3</sup>, I. Kolčić<sup>1</sup>, O. Polašek<sup>1</sup>;*<sup>1</sup>School of Medicine, University of Split, Split, Croatia, <sup>2</sup>Institute for Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, <sup>3</sup>Usher Institute, University of Edinburgh, Edinburgh, United Kingdom.

**Background:** Due to the rigorous genome-wide significance threshold in GWAS studies, causative common low-risk polymorphisms for complex phenotypes may be missed. We propose building a genetic network as post-GWAS approach to provide an insight into the role of host genetics in infectious disease pathogenesis from the 10,001 Dalmatians biobank.

**Materials and methods:** We included 1,998 participants representing isolated island populations and 1,012 participants from mainland population. GWAS with meta-analyses was performed for 11 infection-related phenotypes. Genotypic correlations between each phenotype-associated SNPs from GWAS were used for network construction and enrichment analysis. We also searched for the overlap between correlated infectious phenotypes to find shared core genes and pathways.

**Results:** Three genes reached a genome-wide significance threshold - HA-PLN1 for meningitis in all cohorts, while IPMK for tuberculosis and TTC39B for hepatitis in island cohorts. Genetic network additionally revealed genes

DCN and CORIN to be marginally significant for tuberculosis, DMD for hepatitis and GALNT18 for systemic infections. Pathway analysis also revealed enrichment in genes outside of the immune system that may have plausible functions in various infection-related outcomes.

Conclusions: Network analysis can be a favourable post-GWAS approach, especially to elucidate shared genes among correlated complex phenotypes. Some variants in infectious diseases may be wide-spread, while others could be a result of genetic drift and isolation and possibly even local co-adaptation.

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## P18.059

### Mathematical modelling of NGS data for an alternative classification of paediatric inflammatory bowel disease

*E. Mossotto, B. MacArthur, J. Brodzki, S. Ennis;  
University of Southampton, Southampton, United Kingdom.*

Introduction: Inflammatory bowel disease is a chronic condition typically classified into Crohn's disease, ulcerative colitis and IBD unclassified. Genetics plays an important aetiological role in paediatric disease. We hypothesise that patients with similar genetic mutation profiles may form distinct clusters in a machine learning model that can reflect or add to clinical information/management. We applied machine learning models to whole exome sequencing (WES) data and constructed an alternative classification model for young IBD patients.

Materials and methods: WES data from paediatric IBD cases (n=136) and controls (n=136) were used in model development and processed using customised scripts. Principal component analysis was used as an unsupervised machine learning model for data visualisation and classification.

Results: The inclusion of genomic variants in addition to clinical features in a machine learning model produced patient clusters distinct to those defined using clinical criteria only. Using KGGSeq scores and entropy calculations, we identified genes that discriminated paediatric IBD cases from controls. Furthermore, Jensen-Shannon divergence-based scoring system prioritises known causal genes and generates a restricted list of candidate genes that will be used to build a supervised model for further disease prediction and classification.

Discussion: Our customised gene selection algorithm demonstrates improved case/control discriminatory power above models based on clinical data only and will form the basis for a progressive supervised model.

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## P18.060

### International Breakpoint Mapping Consortium: the 25-year summary of Estonian results

*L. Roht<sup>1</sup>, T. Mõlter-Väär<sup>2</sup>, P. Ilisson<sup>1</sup>, P. Tammur<sup>1</sup>, K. Kuuse<sup>1</sup>, T. Reimand<sup>1,3,4</sup>, K. Õunap<sup>1,3</sup>;  
<sup>1</sup>Tartu University Hospital, United Laboratories, Department of Genetics, Tartu, Estonia,  
<sup>2</sup>Tartu University Hospital, United Laboratories, Department of Genetics, Tallinn, Estonia, <sup>3</sup>Department of Paediatrics, University of Tartu, Tartu, Estonia, <sup>4</sup>Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia, Tartu, Estonia.*

We are participating in International Breakpoint Mapping Consortium (IBMC) since 2015. The aim of this project is to map ~10,000 balanced chromosomal rearrangement breakpoints.

The aim of our study was to retrospectively analyse all cases with apparently balanced translocation or inversion and clinical phenotype since 1990 using the archives of our department (25 years). The collected data consists of clinical information, karyotype, results of chromosomal microarray analysis (CMA), DNA availability and the present status of a person (alive or dead). To all selected cases a request of informed consent was sent out, except dead persons. If the DNA sample was not available and/or CMA was not performed, the person was invited to counselling. We have an approval (244/T-11) from the Tartu University Committee of Ethics for this work.

All selected cases will be sent to Wilhelm Johannsen Centre for Functional Genome Research to be analyzed by Mate-Pair Sequencing to assess whether rearrangements predispose to disease by interrupting or otherwise affecting a specific gene.

We found 325 (2.7 % of all karyotypes from blood) diagnosed cases with apparently balanced translocation or inversion. Most of these investigated patients were clinically healthy, except infertility problems and therefore we did not include them. In 35 cases (10.8 % of diagnosed cases) clearly abnormal phenotype was noticed in addition to balanced aberration. Presently in 16 cases we have collected all needed information and material, which will

be investigated further.

In conclusion: abnormal phenotype and apparently balanced aberrations occur seldom in clinical practice in Estonia.

## P18.061

### Graphical tools for estimating family relationships

*I. Galván-Femenía<sup>1,2</sup>, J. Graffelman<sup>3</sup>, R. De Cid<sup>2</sup>, C. Barceló-i-Vidal<sup>1</sup>;*

*<sup>1</sup>Department of Computer Science, Applied Mathematics and Statistics, Universitat de Girona, Girona, Spain, <sup>2</sup>Diseases-GCAT lab group, Institute of Predictive and Personalized Medicine of Cancer (IMPc) – Germans Trias Health Research Institute (IGTP), Can Ruti Campus Badalona, Barcelona, Spain, <sup>3</sup>Department of Statistics and Operations Research, Universitat Politècnica de Catalunya, Barcelona, Spain.*

Introduction: Estimating family relationships is a quality control step in Genome Wide Association Studies. Statistical methods as logistic regression require each individual to be independent. If there are any close relatives in the sample, the statistical models can generate misleading conclusions. Thus, identifying family relationships in genetic databases is recommended. Material and Methods: The GCAT-Genomes for Life Cohort Study of the Genomes of Catalonia Project is based on the study of the role of genomic and epigenomic factors in the development of cancer and chronic diseases in the Catalonian population. Currently 3500 blood samples have been genotyped by SNP-arrays methods for further analysis. In this contribution, we analyze these samples with the aim of identifying family relationships. Identity by state (IBS) and identity by descent (IBD) alleles are considered for this purpose. We summarize the most used graphical methods in relatedness research and present two additional tools from the field of compositional data analysis.

Results: Several related pairs are expected to be detected in the GCAT database by plotting the mean versus the standard deviation of IBS alleles and by plotting the proportions of sharing 0, 1 or 2 IBS alleles. Plotting the probabilities of sharing 0, 1 or 2 IBD alleles is used to confirm the supposed family relationships. The graphical tools used in compositional data analysis (ternary diagrams and the isometric logratio plots) mainly confirm the expected inferred relationships.

Conclusions: Graphics used in compositional data analysis such as ternary diagrams and logratio plots are useful in relatedness research.

## P18.062

### Dissection of three idiopathic pulmonary fibrosis susceptibility regions using targeted next-generation sequencing for fine mapping

*J. M. Lorenzo Salazar<sup>1</sup>, S. F. Ma<sup>2</sup>, M. Acosta-Herrera<sup>1,3</sup>, Y. Huang<sup>2</sup>, R. Vij<sup>2</sup>, J. Oldham<sup>2</sup>, I. Noth<sup>2</sup>, C. Flores<sup>1,3,4</sup>;*

*<sup>1</sup>Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain,*

*<sup>2</sup>Pulmonary & Critical Care Medicine, University of Chicago, Chicago, IL, United States,*

*<sup>3</sup>CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain,*

*<sup>4</sup>Applied Genomics Group (G2A), Section of Genetics, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, Tenerife, Spain.*

Introduction: Idiopathic pulmonary fibrosis (IPF) is a low incidence, devastating disease with unknown etiology and high mortality. Several studies, including one genome-wide association study (GWAS) completed by our group, have revealed common and rare variants in >15 genes that firmly associate with IPF susceptibility. In order to provide further delineation of the likely causal variants, here we performed a fine-mapping study of the three loci (11p15.5, 14q21.3, and 17q21.31) achieving significance in our GWAS. Materials and Methods: We used a custom-designed target capture Sure-Select XT2 (Agilent) kit and the HiSeq2500 (Illumina) instrument in 181 European-Americans with IPF. Variant sites were identified using a custom GATK-based NGS analysis pipeline. Association testing was performed using unrelated European individuals (n=501) from The 1000 Genomes Project as controls.

Results and Conclusions: Approximately  $10^7$  reads per sample were generated with an average of 100X depth coverage across the region of interest (1.7 Mb). A total of 16,253 variant sites were identified but association tests used genotypes from the 10,245 biallelic single nucleotide polymorphisms with call rates >95%, adjusting for five principal components to account for population stratification (lambda=1.001). Despite the reduced sample size, we identified 36 variants reaching genome-wide significance ( $p < 5 \times 10^{-8}$ ), including one previously identified in the promoter region of *MUC5B* gene (rs35705950), and several other novel susceptibility variants.

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## P18.063

## Improved imputation accuracy with population-specific SNP array and reference panel in Japanese population

Y. Kawai, T. Mimori, K. Kojima, F. Katsuoka, J. Yasuda, M. Yamamoto, M. Nagasaki; Tohoku University, Sendai, Japan.

**Introduction:** Genotype imputation is a key step in genome-wide analysis in human genetics. Accuracy and coverage of imputed variants depend on the selection of SNP array for genotyping as well as the choice of the haplotype reference panel in imputation process.

We developed a new SNP array (Japonica array) optimized for Japanese population and evaluated the imputation accuracy of this array with various reference panels.

**Materials and Methods:** Whole-genome genotype imputation was conducted for the genotypes obtained from 131 individuals by Japonica array and other SNP arrays with cosmopolitan (international 1000 genomes) and population-specific (1070 whole genomes of Japanese individuals) panels. The imputation accuracy and coverage were computed by comparing the imputed genotype with genotypes obtained by whole genome sequencing. **Results:** Better imputation performance was obtained for the Japonica array with the 1KJPN panel compared with other combinations of SNP array and reference panel. The genomic coverage of imputed genotypes ( $r^2 > 0.8$ ) with the Japonica array reached around 97% and 67% for common (MAF > 5%) and low-frequency SNPs (0.5% < MAF ≤ 5%), respectively.

**Conclusions:** A combination of population-specific SNP array with dense haplotype collection improved both imputation accuracy and coverage.

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## P18.064

## Investigating the genetic control of kidney function of individuals in the Scottish population

R. F. Oppong<sup>1</sup>, O. Devuyst<sup>2</sup>, C. Hayward<sup>3</sup>, P. Navarro<sup>3</sup>, C. S. Haley<sup>3,4</sup>, S. Knott<sup>1</sup>; <sup>1</sup>IEB, SBS, University of Edinburgh, Edinburgh, United Kingdom, <sup>2</sup>ZIHP, University of Zurich, Zurich, Switzerland, <sup>3</sup>MRC IGMM, University of Edinburgh, Edinburgh, United Kingdom, <sup>4</sup>The Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom.

Measures of urine electrolytes provide useful information on kidney function in people and are used routinely by clinicians as biomarkers of kidney damage. Genome-wide association scans to identify significant loci associations in these traits will give some indication of the underlying genetics which potentially will provide useful insights into kidney disease aetiology. We therefore performed a mixed model GWAS on 2934 individuals which formed part of a larger cohort of individuals genotyped in the Generation Scotland: Scottish Family Health Study. We analysed 8 phenotypes consisting of measures of urine concentrations of sodium, potassium, chlorides, calcium, glucose, phosphorus, magnesium, and urine osmolarity. We further estimated whole genome and regional heritabilities for these phenotypes using GCTA and REACTA.

We report a GWAS significant SNP (rs4574243, p-value = 9.378709e-09) close to the RBMS3 gene on chromosome 3 associated with urine calcium concentration. The remaining traits had significant SNP associations at a suggestive level (p-value < 1e-06) but not reaching GWAS significance. The regional heritability results in most of the traits mirrored their GWAS results by showing significant estimates on chromosomes with GWAS hits. The heritability estimates were very low for all the traits suggesting that these traits are under modest genetic control.

The SNP hits identified for these traits lie within areas of the genome that have genes close by (<1Mb) which presents a useful avenue to be explored in future studies. There have been very sparse GWAS reports on these traits which make these findings uniquely important.

## P18.066

## Food and pathogen adaptations: tracing the spread of lactase persistence and human African trypanosomiasis resistance into southwestern Africa

J. C. Pinto<sup>1</sup>, S. Oliveira<sup>1,2</sup>, S. Teixeira<sup>1</sup>, D. Martins<sup>3</sup>, A. M. Fehn<sup>1,4</sup>, T. Aço<sup>5</sup>, M. Gayà-Vidal<sup>1</sup>, J. Rocha<sup>1,2</sup>;

<sup>1</sup>CIBIO/InBIO- Research Center in Biodiversity and Genetic Resources, Vairão, Porto, Portugal, <sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal, <sup>3</sup>ISCED/Huila: Instituto Superior de Ciências da Educação, Lubango, Angola, <sup>4</sup>Max Planck Institute for the Science of Human History, Jena, Germany, <sup>5</sup>CEDO: Centro de Estudos do Deserto, Namibe, Angola.

**Introduction:** We investigated the frequency distribution and haplotype diversity of APOL1 and LCT variants associated with human African trypanosomiasis (HAT) resistance and lactase persistence (LP), respectively, in

populations from southern Angola to trace the spread of these genetic adaptations into southwestern Africa.

**Materials and Methods:** We resequenced two fragments of the LCT-enhancer and the APOL1 gene and genotyped flanking STRs in six groups from the Angolan Namib with different subsistence traditions, and in other populations from Africa and Europe for comparative purposes. The age and selection coefficient of these variants were estimated.

**Results:** LP in the Angolan Namib is represented by the -14010\*C allele, which is associated with a predominant haplotype shared with other southern and eastern African populations. While LP was more frequent in foragers than in pastoralists, the frequencies of the two APOL1 variants (G1 and G2) did not differ between the two groups. The G1 allele is mostly associated with a single widespread haplotype. The G2 allele is linked to several haplotypes that are related to haplotypes found in African Bantu-speaking populations. The putatively archaic G3 variant displayed more intra-allelic diversity in Africa than in Europe.

**Conclusions:** The LP adaptation was carried to southern Africa from eastern Africa, probably by non-Bantu speaking pastoralists, although we could not confirm a direct link with groups speaking Khoe-Kwadi family languages. The presence of APOL1 variants G1 and G2 is linked to the Bantu expansions. Our results suggest that the G3 variant was retained in modern humans by incomplete lineage sorting.

## P18.067

## GATA3 gene involvement in leprosy susceptibility in Brazilian population

A. P. Latini, P. Medeiros, K. B. Valezi, W. L. da Silva; Instituto Lauro de Souza Lima, Bauru, Brazil.

Leprosy is a disease caused by *Mycobacterium leprae*. The disease outcome is a complex trait and the host-pathogen-environment interaction defines the emergence of the disease. Human genetic risk factors are definitely associated to leprosy. The chromosomal region 10p13 was linked to leprosy and the *GATA3* gene is a strong candidate to be part of this association. Therefore, we tested if SNPs at *GATA3* are associated to leprosy risk.

The genotyping was made by allelic discrimination using TaqMan SNP assays (Applied Biosystems). All association analyses were conducted applying logistic regression model using R software.

We tested seven tag SNPs in a population from Mato Grosso State, Brazil (411 cases and 357 controls). The A allele of rs10905284 marker was associated with leprosy resistance for CA genotype (OR 0.65; 95%CI 0.47-0.90; p=0.0099) and for A allele carriers (OR 0.67; 95%CI 0.50-0.92; p=0.0117). Then, we tested this association in another Brazilian population from São Paulo State (511 cases and 380 controls). The association was confirmed for A allele carriers (OR 0.67; 95%CI 0.48-0.92; p=0.0151), heterozygous CA (OR 0.69; 95%CI 0.49-0.97; p=0.0344), and for homozygous AA (OR 0.61; 95%CI 0.40-0.93; p=0.0201). A significant association was also observed for the combined population for the CA (OR 0.67; 95%CI 0.53-0.85; p=0.0009) and AA (OR 0.67; 95%CI 0.50-0.89; p=0.0056) genotypes; A carriers (OR 0.67; 95%CI 0.53-0.84; p=0.0004), and for the A allele (OR 0.81; 95%CI 0.66-0.98; p=0.0341).

Thus, we demonstrated for the first time a *GATA3* role in the genetic susceptibility for leprosy.

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## P18.068

## Age-related Macular Degeneration: an overview of the susceptibility genes in the Italian population

C. Strafella<sup>1</sup>, G. Longo<sup>1</sup>, R. Cascella<sup>1,2</sup>, L. T. Marsella<sup>1</sup>, P. Borgiani<sup>1</sup>, F. Viola<sup>3</sup>, C. M. Eandi<sup>4</sup>, F. Sangiulio<sup>1</sup>, G. Novelli<sup>1</sup>, A. Cusumano<sup>5</sup>, G. Staureghini<sup>6</sup>, F. Ricci<sup>5</sup>, E. Giardina<sup>1,7</sup>;

<sup>1</sup>University of Rome Tor Vergata, Rome, Italy, <sup>2</sup>Emotest Laboratory, Pozzuoli, Italy, <sup>3</sup>U.O. Oculist Foundation IRCCS "Cà Granda" Maggiore General Hospital, Milan University, Milan, Italy, <sup>4</sup>Department of Clinical Physiopathology, Eye Clinic, University of Turin, Turin, Italy, <sup>5</sup>UOSD Patologia retinica Fondazione PTV "Policlinico Tor Vergata", Rome, Italy, <sup>6</sup>Eye Clinic, Department of Clinical Science "Luigi Sacco", Sacco Hospital, University of Milan, Milan, Italy, <sup>7</sup>Molecular Genetics Laboratory UILDM, Santa Lucia Foundation, Rome, Italy.

Age-related Macular Degeneration (AMD, OMIM #610149) is the progressive degeneration of the macula and is responsible of the low vision in people aged >55 years old. The disease leads to the gradual loss of central vision and the strong impairment of the ordinary activities.

AMD is a multifactorial disease that involves both environmental (age, smoking, diet, familiarity) and genetic factors (susceptibility loci). In particular, CFH (p=2.7\*10-15), ARMS2 (p=9.9\*10-13) and IL-8 (p=4.15\*10-5) genes are the major contributors to the genetic susceptibility in the Italian population. GWASs conducted in European and Asian populations, identified 13 novel

genes (TIMP3, LIPC, APOE, VEGFA, IER3-DDR1, B3GALTL, TGFBR1, ADAM, COL10A1, CETP, RAD51B, SLC16A8, COL8A1) potentially involved in the development and progression of AMD. These genes have been analyzed in a cohort of 1512 Italian subjects (cases=712, controls=800), in order to set up a genetic risk panel representative of our population. The genotyping analysis was performed by TaqMan assay and 7500 Fast Real Time PCR device and pointed out the association of TIMP3 (rs5749482 C/G; p=3,07823\*10-07 OR C=1.64, 95% CI:1.3-2.0), RAD51B (rs8017304 A/G; p=0.031 OR G=1.2, 95% CI:1.0-1.3), SLC16A8 (rs8135665 C/T; p=4,59283\*10-6 OR T=1.4, 95% CI:1.2-1.7), COL8A1 (rs13081855 G/T; p=1,73\*10-04 OR T=1.6, 95% CI:1.2-2.0), VEGFA (rs943080 C/T; p=0.003 OR T=1.2, 95% CI:1.0-1.3) with AMD in our cohort. These genes, together with CFH, ARMS2 and IL-8, represent a first overview of the genetic contribution to the disease susceptibility in Italian population. These results will be then combined with environmental factors to generate a population-specific "risk platform" for AMD.

#### P18.069

##### The *MC1R* Gene and Youthful Looks

*F. Liu*<sup>1,2</sup>, *M. A. Hamer*<sup>2</sup>, *J. Deelen*<sup>3</sup>, *J. S. Lall*<sup>4</sup>, *L. Jacobs*<sup>2</sup>, *D. van Heemst*<sup>3</sup>, *P. G. Murray*<sup>4</sup>, *A. Wollstein*<sup>2,5</sup>, *A. J. M. De Craen*<sup>3</sup>, *H. Uh*<sup>3</sup>, *C. Zeng*<sup>4</sup>, *A. Hofmann*<sup>2</sup>, *A. G. Uitterlinden*<sup>2</sup>, *J. Houwing-Duistermaat*<sup>3</sup>, *L. M. Pardo*<sup>2</sup>, *M. Beekman*<sup>3</sup>, *P. E. Slagbloom*<sup>3</sup>, *T. Nijsten*<sup>2</sup>, *M. Kayser*<sup>2</sup>, *D. A. Gunn*<sup>4</sup>;

<sup>1</sup>Beijing Institute of Genomics, Beijing, China, <sup>2</sup>Erasmus MC University Medical Center, Rotterdam, Netherlands, <sup>3</sup>Leiden University Medical Center, Leiden, Netherlands,

<sup>4</sup>Colworth Science Park, Sharnbrook, United Kingdom, <sup>5</sup>University of Munich, Planegg-Martinsried, Germany.

Looking young for one's age has been a desire since time immemorial. This desire is attributable to the belief that appearance reflects health and fecundity. Indeed, perceived age predicts survival and associates with molecular markers of aging such as telomere length. Understanding the underlying molecular biology of perceived age is vital for identifying new aging therapies amongst other purposes, but studies are lacking thus far. As a first attempt, we performed genome-wide association studies (GWASs) of perceived facial age and wrinkling estimated from digital facial images by analyzing over 8 million single nucleotide polymorphisms (SNPs) in 2,693 elderly Dutch Europeans from the Rotterdam Study. The strongest genetic associations with perceived facial age were found for multiple SNPs in the *MC1R* gene ( $p<1\times 10^{-7}$ ). This effect was enhanced for a compound heterozygosity marker constructed from four pre-selected functional *MC1R* SNPs ( $p=2.69\times 10^{-12}$ ), which was replicated in 599 Dutch Europeans from the Leiden Longevity Study ( $p=0.042$ ) and in 1,173 Europeans of the TwinsUK Study ( $p=3\times 10^{-3}$ ). Individuals carrying the homozygote *MC1R* risk haplotype looked on average up to two years older than non-carriers. This association was independent of age, sex, skin color, sun-damage (wrinkling, pigmented spots), and persisted through different sun-exposure levels. Hence, a role for *MC1R* in youthful looks independent of its known melanin synthesis function is suggested. Our study uncovers the first genetic evidence explaining why some people look older for their age, and provides new leads for further investigating the biological basis of how old or young people look.

#### P18.070

##### Variants in *MC1R* gene are involved in neurodegenerative diseases in Spanish population

*G. Tell-Martí*<sup>1,2</sup>, *J. A. Puig-Butille*<sup>3,2</sup>, *M. Ezquerre*<sup>4,5</sup>, *V. Alvarez*<sup>6</sup>, *J. M. Millán*<sup>7,8</sup>, *M. J. Trujillo-Tiebas*<sup>9,10</sup>, *M. A. Ramos-Arroyo*<sup>11</sup>, *J. Clarimón*<sup>12,5</sup>, *A. Lladó*<sup>13</sup>, *R. Fernández-Santiago*<sup>4,5</sup>, *M. Milà*<sup>3,2</sup>, *S. Puig*<sup>1,2</sup>;

<sup>1</sup>Dermatology Department, Melanoma Unit, Hospital Clinic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain,

<sup>3</sup>Biochemical and Molecular Genetics Service, Hospital Clinic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain, <sup>4</sup>Laboratory of Neurodegenerative Disorders, Department of Clinical and Experimental Neurology, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain,

<sup>5</sup>Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNE), Barcelona, Spain, <sup>6</sup>Laboratorio de Genética, AGC Laboratorio de Medicina. Hospital Universitario Central de Asturias, Oviedo, Spain, <sup>7</sup>Instituto de Investigación Sanitaria Hospital La Fe, IIS-La Fe, Valencia, Spain, <sup>8</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain, <sup>9</sup>Department of Genetics, Health Research Institute-Jimenez Diaz Foundation University Hospital (IIS-FJD), Madrid, Spain, <sup>10</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain, <sup>11</sup>Medical Genetics Department, Complejo Hospitalario de Navarra, IdiSNA, Navarra Institute for Health Research, Irunlarrea 4, 31008, Pamplona, Spain, <sup>12</sup>Memory Unit, Neurology Department, Hospital de Sant Pau (Sant Pau Biomedical Research Institute), Universitat Autònoma de Barcelona, Barcelona, Spain,

<sup>13</sup>Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain.

Introduction: The melanocortin 1 receptor (*MC1R*) gene is a key regulator of skin and hair colour. Certain *MC1R* polymorphisms, which cause a loss

of protein function, have been associated with red hair colour (RHC) phenotype (red hair, fair skin and poor tanning response) and a higher risk of developing skin cancer. Although the *MC1R* protein is mainly expressed in melanocytes, it is also detected in neurons. We have observed that skin cells harbouring loss-of-function *MC1R* polymorphisms showed a deregulation of genes involved in pathways related to neurodegenerative diseases (Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD)).

**Material and Methods:** We performed two case-control studies to elucidate the role of *MC1R* in PD and AD. We sequenced the *MC1R* gene in 870 PD patients, 525 AD patients and 736 controls from Spain. We assessed the role of *MC1R* as a modifier factor on age of onset (AOO) in HD, sequencing *MC1R* in 600 HD patients. We analyzed all non-synonymous *MC1R* variants with a minor allele frequency of at least 0.01.

**Results:** We found evidence that the loss-of-function p.R160W *MC1R* polymorphism is marginally associated with PD (OR=2.10; Bonferroni-corrected P=0.063) and that the p.V92M *MC1R* polymorphism increased risk of AD (OR: 1.97, P= 0.021). Furthermore, another loss-of-function polymorphism (p.R151C) showed an effect on AOO in HD (Bonferroni-corrected P = 0.032), which explains 1.42% of the variance in AOO that cannot be accounted for by the expanded HD allele.

**Conclusion:** Our results suggest that *MC1R* may play a role in neurodegenerative diseases.

#### P18.071

##### Role of *POT1* germline variants in familial melanoma in Spain

*M. Potrony*<sup>1</sup>, *M. Ribera-Sola*<sup>1</sup>, *J. A. Puig-Butille*<sup>2,3</sup>, *V. Iyer*<sup>4</sup>, *C. D. Robles-Espinoza*<sup>4</sup>, *C. Badenas*<sup>2,3</sup>, *P. Aguilera*<sup>1,3</sup>, *C. Carrera*<sup>1,3</sup>, *J. Malvehy*<sup>1,3</sup>, *D. J. Adams*<sup>4</sup>, *S. Puig*<sup>1,3</sup>;

<sup>1</sup>Dermatology Department, Melanoma Unit, Hospital Clinic of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain,

<sup>2</sup>Biochemical and Molecular Genetics Service, Hospital Clinic of Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain, <sup>4</sup>Experimental Cancer Genetics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

**Introduction:** Protection of telomeres 1 (POT1, NM\_015450, 7q31) encodes a protein of the telomeric shelterin complex, which plays an important role in telomere maintenance. Two independent groups identified rare germline variants in POT1 that predispose to melanoma.

**Material and Methods:** POT1 was sequenced by next generation sequencing (NGS) in 183 probands from Spanish melanoma-prone families. Generated libraries, following Fluidigm unidirectional sequencing protocol, were sequenced on an Illumina MiSeq. Reads were aligned to the reference genome (GRCh37) using BWA mem. Variants were called with the GATK Haplotype-Caller and quality filters were set as standard. Variant effect was predicted with the Ensembl Variant Effect Predictor release 70. Missense variants or synonymous variants affecting splicing were sequenced by Sanger sequencing in the families detected for validation.

**Results:** We detected by NGS 11 candidate variants in 13 probands, from the 119 samples that passed the quality control. DNA from patients belonging to 11/13 families was available. We detected by Sanger sequencing 3/9 candidate variants that could be tested in 3 melanoma-prone families: p.Gly404Val (c.1211G>T, ENST00000357628, ExAC frequency=0.014) which is predicted to be probably benign, suggesting it is a polymorphism; p.Ile78Thr (c.233T>C, ENST00000357628, ExAC frequency=0), which was previously detected in a melanoma patient and is predicted to be pathogenic, and finally the p.Lys85Lys (c.255G>A, ENST00000357628, ExAC frequency=1e-05), which is located in a splicing site and furthermore cosegregates with melanoma within the family. Thus 2/119 (1.7%) families carry probably pathogenic variants in POT1.

**Conclusion:** POT1 germline variants play a role in familial melanoma susceptibility in Spain.

#### P18.072

##### A comprehensive genome-wide analysis of melanoma Breslow thickness identifies interaction between *CDC42* and *SCIN* genes

*A. Vaysse*<sup>1,2</sup>, *S. Fang*<sup>3</sup>, *M. Brossard*<sup>1,2</sup>, *Q. Wei*<sup>4</sup>, *W. V. Chen*<sup>3</sup>, *H. Mohamdi*<sup>1,2</sup>, *L. Vincent-Fetita*<sup>5</sup>, *P. Margaritte-Jeannin*<sup>1,2</sup>, *N. Lavielle*<sup>1,2</sup>, *E. Maubec*<sup>1,6</sup>, *M. Lathrop*<sup>7</sup>, *M. Avril*<sup>8</sup>, *C. I. Amos*<sup>9</sup>, *J. E. Lee*<sup>9</sup>, *F. Demenais*<sup>1,2</sup>;

<sup>1</sup>INSERM U946, Paris, France, <sup>2</sup>Université Paris Diderot, Paris, France, <sup>3</sup>MD Anderson Cancer Center (MDACC), Houston, TX, United States, <sup>4</sup>Duke University School of Medicine, Durham, NC, United States, <sup>5</sup>Hôpital Cochin, Université Paris Descartes, Paris, France, <sup>6</sup>Hôpital Avicenne et Université Paris 13, Bobigny, France, <sup>7</sup>McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada, <sup>8</sup>Geisel College of Medicine, Dartmouth College, Lebanon, NH, United States.

Breslow thickness (BT) is a major prognostic factor of cutaneous melanoma, the most fatal skin cancer. The genetic component of BT has only been explored by candidate gene studies with inconsistent results. Our objective

was to uncover the genetic factors underlying BT using a hypothesis-free genome-wide approach.

Our analysis strategy integrated a genome-wide association study (GWAS) for BT followed by pathway analysis of GWAS outcomes using the gene-set enrichment analysis (GSEA) method and epistasis analysis within BT-associated pathways. This strategy was applied to two large melanoma datasets with Hapmap3-imputed SNP data (~1 million SNPs): the French MELARISK study for discovery (966 cases) and the MD Anderson Cancer Center study (1,546 cases) for replication.

While no marginal effect of individual SNPs was revealed through GWAS, three pathways, defined by gene ontology (GO) categories were significantly enriched in genes associated with BT (False Discovery Rate  $\leq 5\%$  in both studies): hormone activity, cytokine activity and myeloid cell differentiation. Epistasis analysis, within each significant pathway, identified a statistically significant interaction between *CDC42* and *SCIN* SNPs ( $P_{\text{interaction}} = 2.2 \times 10^{-6}$ ), which met the overall multiple-testing corrected threshold of  $2.5 \times 10^{-6}$ . These two SNPs (and proxies) are strongly associated with *CDC42* and *SCIN* gene expression levels and map to regulatory elements in skin cells.

In conclusion, this study identified two novel genes influencing Breslow thickness. This finding has important biological relevance since *CDC42* and *SCIN* proteins have opposite effects in actin dynamics, a key mechanism underlying melanoma cell migration and invasion.

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#### P18.073

#### Association between depressive symptoms and insulin resistance is moderated by melatonin receptor 1B gene polymorphisms

K. Haljas<sup>1</sup>, J. M. Lahti<sup>1,2,3</sup>, T. Tuomi<sup>4,2,5</sup>, L. H. Hakaste<sup>4,2,5</sup>, B. Isomaa<sup>6,2</sup>, J. G. Eriksson<sup>7,8,2</sup>, L. C. Groop<sup>9,5</sup>, K. Räikkönen<sup>1</sup>,

<sup>1</sup>Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland, <sup>2</sup>Folkhälsan Research Center, Helsinki, Finland, <sup>3</sup>Collegium for Advanced Studies, University of Helsinki, Helsinki, Finland, <sup>4</sup>Department of Endocrinology, Abdominal Centre, Helsinki University Hospital, Helsinki, Finland, <sup>5</sup>Finnish Institute for Molecular Medicine, University of Helsinki, Helsinki, Finland, <sup>6</sup>Department of Social Services and Health Care, Jakobstad, Finland, <sup>7</sup>Department of General Practice and Primary Health Care, University of Helsinki and Helsinki University Hospital Helsinki, Helsinki, Finland, <sup>8</sup>National Institute for Health and Welfare, Helsinki, Finland, <sup>9</sup>Diabetes and Endocrinology, Department of Clinical Sciences, Lund University Diabetes Centre, Malmö, Sweden.

**Background:** Mechanisms for commonly shown association between depression and type 2 diabetes (T2D) are poorly understood. One possible explanation is disruption in circadian regulation placing melatonin signaling in key role. We set out to test if rs10830963 in MTNR1B gene - encoding for melatonin receptor 2 - that increases T2D risk moderates the association between depressive symptoms, insulin resistance and insulin secretion.

**Methods:** Participants of the PPP-Botnia (N=994) and Helsinki Birth Cohort Study (HBCS; N=1720) underwent oral glucose tolerance test (OGTT) and filled in Mental Health Index (MHI-5) of SF-36 questionnaire to study depressive symptoms at the mean age of 57.5 (SD: 10.7) and 61.5 (SD: 2.9), respectively. rs10830963 was directly genotyped in the PPP-Botnia and its proxy SNP rs1447352 (D': 1.0; r2: 0.4) in the HBCS. We used covariates-adjusted linear regression models to test the associations between the traits.

**Results:** In PPP-Botnia, rs10830963 moderated the association between depressive symptoms and fasting insulin, insulin area under curve (AUC), insulin resistance (HOMA-IR), and insulin sensitivity index (ISI) (p-value for interactions  $< .02$ ). This pattern replicated in the HBCS. Depressive symptoms were significantly associated with higher fasting insulin levels, insulin AUC, HOMA-IR and lower ISI in homozygous individuals for risk allele but not in those who were homozygous for non-risk allele consistently in both cohorts. We did not find any significant interaction on glucose levels or CIR and DI. **Conclusions:** We show that those with depressive symptoms are in greater risk of developing insulin resistance if they carry risk genotype for diabetes in MTNR1B.

#### P18.074

#### MEFV Mutations in Turkish Patients Suffering From Familial Mediterranean Fever

M. Ozdemir<sup>1</sup>, O. Çilingir<sup>1</sup>, H. Aslan<sup>1</sup>, B. D. Aras<sup>1</sup>, S. Arslan<sup>1</sup>, Ö. Kutlay<sup>1</sup>, C. Korkmaz<sup>2</sup>, S. Artan<sup>1</sup>,

<sup>1</sup>Eskişehir Osmangazi University Department of Medical Genetics, Eskişehir, Turkey, <sup>2</sup>Eskişehir Osmangazi University Department of Rheumatology, Eskişehir, Turkey.

MEFV Mutations in Turkish Patients Suffering From Familial Mediterranean Fever

Familial Mediterranean fever (FMF) is an autosomal recessive disorder. Over 200 mutations have been identified in the MEFV gene responsible for

FMF. In the diagnosis of FMF the combination of Tel-Hashomer Clinical Criteria (THCC) molecular methods can be used.

**AIM:** To identify distributions and frequencies of the MEFV gene mutations in patients.

**PATIENTS-METHODS:** The study was carried out on 3709 clinically diagnosed patients. Mutation screening of the MEFV gene was performed by pyrosequencing of exons 2, 3, 5 and 10 in 3709 patients.

**RESULTS:** The results of our study are summarized in the table.

**CONCLUSION:** Exon 10 is the most common site for FMF mutations whereas exon 2, 3 and 5 accounts for 32.13 % of the cases. The most common mutations among Turkish patients are M694V (A>G) and E148Q.

#### P18.075

#### Genetic mitochondrial variation in the population of Transylvania revealed a different genetic diversity compared to the other regions of Romania: an influence of central Europe

F. Raicu<sup>1,2</sup>, S. Schipor<sup>3</sup>, M. Hervella<sup>4</sup>, P. Cianga<sup>5</sup>, R. Popescu<sup>6</sup>, C. Banescu<sup>7</sup>, A. Martinescu<sup>8</sup>, M. Constantinescu<sup>2</sup>, L. Bohilteanu<sup>1</sup>, R. Cocos<sup>1,9</sup>,

<sup>1</sup>“Carola Davila” University of Medicine and Pharmacy, Bucharest, Romania, <sup>2</sup>“Francisc I. Rainer” Anthropological Research Institute, Romanian Academy, Bucharest, Romania,

<sup>3</sup>National Institute of Endocrinology “C. I. Parhon”, Bucharest, Romania, <sup>4</sup>University of the Basque Country, Department of Genetics, Bizkaia, Spain, <sup>5</sup>Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania, <sup>6</sup>“Victor Babes” University of Medicine and Pharmacy, Timisoara, Romania, <sup>7</sup>University of Medicine and Pharmacy Tg. Mures, Tigru Mures, Romania, <sup>8</sup>Ovidius University, Faculty of Medicine, Constanta, Romania, <sup>9</sup>Genome Life Research Center, Bucharest, Romania.

**Introduction:** The aim of this study was to investigate if the population of Transylvania have a stronger Central European influence in comparison to the Romanian population located south east of the Carpathian Arch, considering its particular geography and various historical events such as different migration routes and colonisation events that could be reflected in the mitochondrial genetic diversity.

**Materials and Methods:** We analysed 714 samples originated from Wallachia (n= 226), Dobrudja (n=46), Moldavia (n=235) and Transylvania (n=207). The sequence of mtDNA control region for all samples was determined by sequencing the hypervariable segments HVS I and HVS II using two pair of primers.

Descriptive statistical indexes, the Tajima's D and Fu's FS neutrality tests based on control region sequences were calculated using Arlequin software. In order to visualize the relationships between population in Transylvania and other populations of Europe and Middle East, including the populations in of the other Romanian provinces, Principal Component analysis (PCA) and nonparametric multidimensional scaling (MDS) analyses were performed using SPSS software.

**Results and Conclusions:** Our findings revealed a genetic homogeneity across all Romanian regions with a lower genetic input from Slavic populations compared to neighbouring populations. A closer genetic affinity of population of Transylvania to Central Europe was indicated by both PCA and MDS analyses. Our data suggest that the geographical features of Romania territory probably had a differentiated impact on the shaping of genetic pool in the Romanian regions in the past.

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#### P18.076

#### lme4qtl: an efficient and flexible tool for QTL mapping in related individuals

A. Ziyatdinov<sup>1</sup>, H. Brunel<sup>1</sup>, M. Vázquez-Santiago<sup>2</sup>, A. Martínez-Perez<sup>1</sup>, J. Soria<sup>1</sup>,

<sup>1</sup>Unitat de Genòmica de Malalties Complexes. Institut d'Investigació Biomèdica Sant Pau (IIB-Sant Pau), Barcelona, Spain, <sup>2</sup>Unitat de Trombosis i Hemostàsia. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

**Introduction:** Mixed models have become a widely used method in quantitative genetics to study continuous and discrete traits. There are many existing tools available for mixed models, although they might lack flexibility when a more complex model is sought. The proposed lme4qtl R package is an extension of the lme4 R package, and allows an arbitrary number of correlated random effects with any correlation structure defined by a covariance matrix and/or grouping factors. A model of gene-environment interaction is shown for illustration.

**Materials and Methods:** Three main techniques were implemented. First, a correlated random effect, for example, defined via the double kinship matrix, was introduced into the model via the Cholesky decomposition of the covariance matrix and further update of the incidence matrix (method by Harville and Callanan). Second, the nearest positive definite of a real symmetric matrix was estimated for semidefinite covariance matrices (the al-

gorithm of Higham). Third, necessary constraints on the model parameters were incorporated in the framework of the lme4 package, mainly for the inference using the likelihood-ratio test (LRT) statistics.

Results: Sophisticated models for quantitative trait locus (QTL) mapping become available with the lme4qtl package. The following code shows an aging model, where the polygenic random effect interacts with the environmental factor (age).

```
repmatLmer(BMI ~ AGE + SEX + (1+AGE|ID) + (1+AGE|RID), dat, repmat = list(ID = kin2), weights = w, vcControl = c)
```

Conclusions: The lme4qtl R package allows (generalized) linear mixed models with correlated random effects for QTL mapping.

## P18.077

### A piebald cladistic portray of mitochondrial DNA control region haplogroups in Khyber Pakhtunkhwa, Pakistan

S. Bhatti;

University of Health Sciences, Lahore, Pakistan.

Despite being situated at the crossroad of Asia, Pakistan has gained crucial importance because of its pivotal role in subsequent migratory events. To highlight the genetic footprints in an enigmatic picture of the relative population expansion pattern among four major Pashtun tribes in Khyber Pakhtunkhwa viz., Bangash, Khattak, Mahsuds and Orakzai, the complete mitochondrial control region of 100 Pashtun were analyzed. All Pashtun tribes studied here revealed high genetic diversity; that was comparable to the other Central Asian, Southeast Asian and European populations. The configuration of genetic variation and heterogeneity further unveiled through Multidimensional Scaling, Principal Component Analysis, and phylogenetic analysis. The results revealed that the Pashtun is a composite mosaic of West Eurasian ancestry of numerous geographic origin. They received substantial gene flow during different invasions and have a high element of the Western provenance. The most common haplogroups reported in this study are: South Asian haplogroup M (28%) and R (8%); whereas, West Asians haplogroups are present, albeit in high frequencies (67%) and widespread over all; HV (15%), U (17%), H (9%), J (8%), K (8%), W (4%), N (3%) and T (3%). Herein we linked the unexplored genetic connection between Ashkenazi Jews and Pashtun. The presence of specific haplotypes J1b (4%) and K1a1b1a (5%) point to a genetic connection of Jewish conglomeration with Khattak tribe. This was a result of an ancient genetic influx in the early Neolithic period that led to the formation of a diverse genetic substratum in present day Pashtun.

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## P18.078

### Novel association of bone metabolism genes with the susceptibility to Psoriatic Arthritis

R. Cascella<sup>1,2</sup>, G. Longo<sup>1</sup>, C. Strafella<sup>1</sup>, M. Ragazzo<sup>1</sup>, E. Galli<sup>2</sup>, L. Marsella<sup>1</sup>, F. Sangiulio<sup>1</sup>, G. Novelli<sup>1</sup>, A. Orlando<sup>3</sup>, E. Giardina<sup>4</sup>;

<sup>1,2</sup>Tor Vergata<sup>1</sup> University of Rome, Rome, Italy, <sup>2</sup>Emotest Laboratory, Pozzuoli, Italy,

<sup>3</sup>S. Pietro Fatebenefratelli Hospital, Rome, Italy, <sup>4</sup>Rome, Italy, <sup>4</sup>Anatomic Pathology, Department of Biomedicine and Prevention, "Tor Vergata" University, Rome, Italy, Rome, Italy, <sup>5</sup>"Tor Vergata" University of Rome; Molecular Genetics Laboratory UILDM, Santa Lucia Foundation, Rome, Italy, Rome, Italy.

Psoriatic Arthritis (PsA, #607507) is a multifactorial inflammatory arthropathy. Although several inflammatory, immunologic and epidermal differentiating genes (HLA-Cw\*0602, LCE3B, LCE3C, TRAF3IP2, ILs, RUNX3, TNF $\alpha$ , SLC22A5) are implicated in PsA etiopathology, the contribution of bone metabolism genes to the disease development is totally unknown. We therefore focused our attention to the 5q31 locus, which contains several PsA-associated genetic variants as well as a number of genes involved in the bone metabolism. On this subject, we studied the genomic variability of the 5q31 locus with a special focus to the region falling within 132,674,018-132,737,310 bp. From the in-silico study of the region, 4 SNPs (rs2227282, rs2285700, rs10062446, rs2897442) were genotyped in 1526 subjects (PsA=500, PsV=426, controls=600). The rs2227282 was localized in IL-4, while the rs2285700, rs10062446, rs2897442 were positioned in KIF3A. All of them resulted associated with PsA ( $p=2.09601*10^{-5}$ ,  $p=3*10^{-3}$ ,  $p=5*10^{-3}$ ,  $p=1.2*10^{-4}$ , respectively) but not with PsV. The allele architecture analysis showed that the haplotype G-A-A-A was of risk, while the haplotype C-C-T-G was protective for PsA. We then extended our research to the KIF3A promoter, identifying other two PsA-associated SNPs (rs2277065,  $p=0.001$  and rs2277066,  $p=0.004$ ). Although the expression analysis was not possible, immunohistochemistry studies showed a strong signal of KIF3A in synovial and cartilaginous tissues. Interestingly, KIF3A and IL-4 are known to participate to osteogenic and remodelling bone activities. Our results pave

the path for the characterization of a cluster of PsA-associated genes within the 5q31 locus, in which immune/inflammatory and bone metabolism genes may interact into a unique etiopathogenetic pathway.

## P18.079

### Identifying genomic determinants and context dependencies of human germline mutation using very rare variants

S. Zöllner, J. Carlson, BRIDGES Consortium, J. Li;

University of Michigan, Ann Arbor, MI, United States.

Mutation is a fundamental biological process driving evolution and generating new disease mutations. Mutation rates depend on genomic context and adjacent nucleotides, but the exact effect of these predictors is unknown. Analyzing de novo variants provides an insufficient number of observations to obtain high-resolution estimates and estimates from species divergence are biased by selection and biased gene conversion. Here, we present a novel approach to comprehensively assess predictors of mutability.

We collect a dataset of over 36 million high quality singleton variants from 3765 whole-genome sequences. These singletons arose very recently in the population, and are thus largely unaffected by confounding evolutionary factors. Based on these variants, we develop statistical models to predict the effect of genomic context and adjacent nucleotides on the mutation density and mutation rates at a single-base resolution.

We find strong evidence for heterogeneity in the mutation rates of short sequence motifs up to 4bp upstream and downstream from the mutating site and estimate up to 60-fold difference between non-CpG motifs within a single mutation category. This heterogeneity provides insight into the biological processes creating germline mutations. The distribution of short motifs alone explains over 70% of variance in singleton density, implicating sequence context as a key determinant of mutability. The single-base mutation rate is further mediated by local genomic features, such as replication timing and histone marks. We also demonstrate that site-specific mutation rates can provide evidence for pathogenicity that is largely independent of existing measures.

## P18.080

### Positive selection at ABCA12.

R. Sirica<sup>1</sup>, M. Buonaiuto<sup>1</sup>, D. Antonini<sup>2</sup>, L. Sticco<sup>3</sup>, O. Guardiola<sup>1</sup>, C. Missero<sup>2</sup>, G. D'Angelo<sup>2</sup>, Q. Ayub<sup>4</sup>, C. Tyler-Smith<sup>4</sup>, V. Colonna<sup>1</sup>;

<sup>1</sup>National Research Council, Institute of Genetics and Biophysics, Napoli, Italy,

<sup>2</sup>University of Naples, "Federico II", Napoli, Italy, <sup>3</sup>National Research Council, Institute of Protein Biochemistry, Napoli, Italy, <sup>4</sup>Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

ABCA12 is a lipid carrier protein expressed in keratinocytes. It is essential for forming skin lipid barrier and is down regulated by UV radiation. Its mutations cause a rare recessive disorder characterized by accumulation of intracellular lipids, disruption of lipid barrier and formation of dry, scaly skin (ichthyosis). We found a signal of positive selection in non-Africans associated with the derived allele of an intronic variant (rs10180970) which is the most differentiated variant between Africans and non-Africans in this gene. To elucidate the environmental pressure responsible for this signal and investigate possible connections with ichthyosis, we analysed data obtained from 56 ancient hominins. The derived allele at rs10180970 is observed for the first time 45,000 years ago (ya) in a heterozygous state in one ancient DNA sample from Asia that shares a 27 kb haplotype with modern Eurasians and is homozygous in 90% Neolithic and Bronze age Eurasians. The high coverage archaic hominins (Neanderthal and Denisovan) older than 50,000 ya are homozygous for the ancestral allele. Preliminary data suggests an increase of ABCA12 expression between populations significantly associated with the T allele at rs10180970. Non expression condition of the gene is seen in Ichthyosis, therefore we are investigating its possible connection with rs10180970. Study of variability through time confirms signal of positive selection showing an increase in frequency for the derived T allele. We are, then, conducting cell assays to understand the role of this variant and to elucidate its part in disease onset.

## P18.081

### Young population profiling for NCDs risk assessment

A. Levitsky, G. Kurochkin;

State University of Medicine and Pharmacy Nicolae Testemitanu, Chisinau, Moldova, Republic of.

In presented research, non-communicable diseases in young population for diseases risk assessment are the aim. There were collected over 3000 samples of young people, with no disease related clinical evidences. About 1500

samples from young patients with established diagnoses as obesity, T2D, dyslipidemia and arterial hypertension are to be collected. It was created the database of patients inquirers, elaborated based on STEPS survey of NCDs in Moldova, comprising over 250 parameters from each of the participants. There was used the approach to identify SNPs related to the biochemical parameters and SNPs with established expression QTL association that potentially can be more informative biomarkers in disease risk assessment. In the first instance, SNPs related to Lipid parameters from GWAS Catalog were obtained. The results related to research of treatment effects, complications and non-European ancestry individuals were removed. There were determined 298 individual SNPs, separated in 3 groups, by reported genes: 1SNP - 1 trait, 1SNP  $\geq$  2 traits,  $\geq$  2 SNPs - 1 trait. None of the SNPs located on chromosome 21, X and Y were identified. Also, only one SNP, located on chromosome 13, associated with BRCA2 gene was present. All selected SNPs were searched through eQTL Browser returning three results (rs10889353, rs2338104, rs646776, p-value < 10<sup>-5</sup>) associated with DOCK7, KCTD10 and CELSR2 genes, respectively. These SNPs will be used to evaluate their capacity of risk assessment at young people, by genotyping based on TaqMan technique.

The research was supported by two institutional grants, 11.817.09.21A and 15.817.04.42A (2011-2014, 2015-2018, respectively).

#### P18.082

#### Polymorphism in FTO, TP53 and Leptin Gene, Dietary Intake and Metabolic Biomarkers in Obese Malaysian Adults

P. Tan<sup>1</sup>, F. Amini<sup>2</sup>, S. R. Mitra<sup>1</sup>;

<sup>1</sup>Nottingham University, Semenyih, Malaysia, <sup>2</sup>UCSI University, Kuala Lumpur, Malaysia.

**Introduction:** Gene variation can explain individual differences in response to dietary interventions on weight management and might contribute to the success of interventions. This study aims to identify polymorphism of selected genes, metabolic biomarkers and dietary intake in obese Malaysian adults. **Method:** Malaysian adults with BMI>23, were genotyped for *FTO* (rs9939609), *TP53* (rs1800371) and *Leptin* (rs7799039) using PCR-RFLP. Dietary recall was used to collect information on detailed habitual food intake. Biochemical parameters were analyzed by standard procedures. **Result:** Twenty three participants (17 females and 6 males) have been recruited so far. Average BMI was 31.4 $\pm$ 6.7 kg/m<sup>2</sup>. Mean age was 30.1 $\pm$ 10. The average total blood cholesterol of 5.7 $\pm$ 1.1 mmol/L; blood triglycerides 1.4 $\pm$ 1.2 mmol/L; HDL cholesterol 1.6 $\pm$ 0.5 mmol/L and LDL cholesterol 3.3 $\pm$ 1.4 mmol/L was observed. The average total calorie intake per day was 2013  $\pm$  602 kcal. Fasting glucose level for GA carrier in rs7799039 was 5.6 mmol/L, significantly higher than 4.6 mmol/L for GG (p=0.04). The same SNP, mean for dietary glucose intake was significantly higher in GA (70.8g) compared to GG (6.8g) (p=0.001). Systolic blood pressure was significantly higher in TT carriers in rs9939609 (124 $\pm$ 14.8 mmHg) compared to TA (109 $\pm$ 4.7 mmHg) (p=0.01). Currently the study participants are undergoing dietary and lifestyle changes in a multidisciplinary weight management programme. **Conclusion:** Our analysis showed hyper-lipaemic blood in all subjects. *Lep* rs7799039 (GA) was associated with higher fasting glucose level and more intake of dietary glucose. *FTO* rs9939609 (TT) was associated with higher systolic blood pressure. The project is funded by UNMC and UCSI Universities.

#### P18.084

#### Polymorphisms in IRF6 and 17q22 loci are risk factors for nonsyndromic orofacial clefts in Slovak population

J. Salagovic<sup>1</sup>, L. Klimcakova<sup>1</sup>, M. Zabavnikova<sup>2</sup>, J. Behunova<sup>3,4</sup>, T. Hudakova<sup>1</sup>, J. Fedele<sup>5</sup>, A. Molnarova<sup>5</sup>, L. Podracka<sup>3,6</sup>;

<sup>1</sup>Department of Medical Biology, Faculty of Medicine, P.J. Safarik University, Kosice, Slovakia, <sup>2</sup>Department of Plastic, Reconstructive and Aesthetic Surgery, University Hospital, Kosice, Slovakia, <sup>3</sup>1st Department of Pediatrics, Faculty of Medicine in P.J. Safarik University and Children's University Hospital, Kosice, Slovakia, <sup>4</sup>Medizinische Universität Wien, Institut für Medizinische Genetik, Wien, Austria, <sup>5</sup>Department of Plastic, Reconstructive and Aesthetic Surgery, Faculty of Medicine in Comenius University and University Hospital, Bratislava, Slovakia, <sup>6</sup>1st Department of Pediatrics, Faculty of Medicine in Comenius University and Children's University Hospital, Bratislava, Slovakia.

**Introduction:** Nonsyndromic orofacial clefts are one of the most common birth defects, having multifactorial etiology involving both genetic and environmental factors. Linkage analyses and genome-wide association studies have identified several genomic susceptibility regions for this orofacial anomaly. Genetic predisposition to nonsyndromic orofacial clefts is ethnicity-dependent, and the genetic basis of susceptibility to this birth defect likely varies among populations.

In the present study 4 single nucleotide polymorphisms that have been pre-

viously identified by genome-wide association studies in other populations were analysed for an association with nonsyndromic orofacial clefts in a Slovak population.

**Materials and Methods:** Nucleotide variants rs642961 in IRF6, rs987525 in 8q24, rs7078160 in 10q25 and rs227731 in 17q22 were genotyped in 199 patients with nonsyndromic orofacial clefts and 157 unaffected controls. All variants of interest were analyzed by high-resolution melting analysis after the real-time PCR on Eco Real-Time PCR System.

**Results:** Significant associations with nonsyndromic orofacial cleft risk were observed for rs642961 and rs227731. Polymorphism rs642961 increased the risk when analyzed under a dominant model (GA + AA vs. GG: OR=1.66; 95% CI 1.06-2.60). Polymorphism rs227731 was significantly associated with an increased risk under a recessive model (CC vs. CA + AA: OR=1.71; 95% CI 1.03-2.83).

**Conclusions:** Our findings suggest that polymorphisms rs642961 in IRF6 and rs227731 in 17q22 are associated with an increased risk of nonsyndromic orofacial clefts and thus might act as a risk factor in Slovak population.

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#### P18.085

#### Coding variants in the MGP gene are involved in osteoarthritis of the hand

C. G. Boer<sup>1</sup>, W. den Hollander<sup>2</sup>, D. Hart<sup>3</sup>, M. S. Yau<sup>4,5</sup>, Y. F. M. Ramos<sup>2</sup>, S. Metrustry<sup>3</sup>, L. Broer<sup>1</sup>, J. Deelen<sup>2</sup>, M. Beekman<sup>2</sup>, L. A. Cupples<sup>6</sup>, F. Rivadeneira<sup>4</sup>, M. Kloppenburg<sup>2</sup>,

M. Peters<sup>1</sup>, T. D. Spector<sup>3</sup>, A. Hofman<sup>1</sup>, P. E. Slagboom<sup>2</sup>, R. G. H. N. Nelissen<sup>2</sup>, A. G. Uitterlinden<sup>1</sup>, D. T. Felson<sup>4,7</sup>, A. Valdez<sup>8</sup>, I. Meulenbelt<sup>2</sup>, J. B. J. van Meurs<sup>1</sup>;

<sup>1</sup>Erasmus MC, Rotterdam, Netherlands, <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>King's College London, London, United Kingdom, <sup>4</sup>Boston University School of Medicine, Boston, MA, United States, <sup>5</sup>Harvard Medical School, Boston, MA, United States, <sup>6</sup>Boston University School of Public Health, Boston, MA, United States,

<sup>7</sup>University of Manchester, Manchester, United Kingdom, <sup>8</sup>School of Medicine, University of Nottingham, Nottingham, United Kingdom.

**Introduction:** Osteoarthritis of the hand (OA) is to a large extend genetically determined. However only one locus has been found to be associated to hand OA. We aimed to identify novel genes and pathways involved in the aetiology of hand OA, by performing a genome-wide association study.

**Materials and methods:** We have performed a GWAS on 12,784 individuals (discovery:8743, replication:4011) using a quantitative bilateral hand OA phenotype, summing all KL-scores of the hand joints for both hands (min: 0, max: 120). RNA-sequencing of 96 human primary chondrocytes was used for allele specific expression analysis of GWAS top hits.

**Results:** After discovery, replication and joint-meta-analysis one SNP reached genome-wide significance (P=1.8\*10<sup>-15</sup>). The risk allele of this variant associates with a higher KL-sum score ( $\Delta$ KL-sum=8) compared to the reference. The variant is located near the Matrix-Gla protein (MGP) gene, which is a calcification inhibitor, and is in high ( $r^2 > 0.8$ ) linkage disequilibrium with a nonsynonymous and a 5'UTR variant in MGP. We found MGP to be highly expressed in human chondrocytes. Additionally these variants cause for allelic imbalance of MGP gene expression in human chondrocytes. The risk allele was consistently lower expressed compared to the reference allele (P<0.001).

**Conclusions:** We identified coding variants in MGP, which are genome-wide-associated to hand OA, and cause for decreased MGP expression. Carriers of the risk alleles seem more prone to develop articular cartilage calcification, a well described hallmark of OA. MGP function is dependent on vitamin-K, indicating vitamin K as a potential therapeutic target for OA.

#### P18.086

#### Identification of new genetic loci and environmental factors associated with parathyroid hormone levels

A. Boljat<sup>1</sup>, I. Gunjača<sup>1</sup>, N. Vidan<sup>1</sup>, V. Torlak<sup>2</sup>, D. Brdar<sup>2</sup>, A. Punda<sup>2</sup>, B. Ložić<sup>3</sup>, V. Boraska Perica<sup>4</sup>, O. Polašek<sup>4</sup>, M. Barbalić<sup>4</sup>, C. Hayward<sup>5</sup>, T. Zemunić<sup>1</sup>;

<sup>1</sup>Department of Medical Biology, University of Split, School of Medicine, Split, Croatia,

<sup>2</sup>Department of Nuclear Medicine, University Hospital Split, Split, Croatia, <sup>3</sup>Department of Pediatrics, University Hospital Split, Split, Croatia, <sup>4</sup>Department of Public Health, University of Split, School of Medicine, Split, Croatia, <sup>5</sup>Institute for Genetics and Molecular Medicine, Western General Hospital, University of Edinburgh, Edinburgh, United Kingdom.

**Introduction:** Parathyroid glands control calcium levels in blood through parathyroid hormone (PTH) regulation. Variations in PTH levels are under genetic control and influenced by environmental factors. The aim of this study was to identify genetic variants and environmental factors underlying parathyroid function by studying PTH levels in healthy individuals. To our knowledge, this is the first study that examines PTH using genome-wide

data.

Methods: PTH was measured in blood plasma of 1012 participants from Split, Croatia (aged 18-85). To identify variants associated with PTH, genome-wide association study was performed using linear regression analysis and an additive model, with adjustments for age, gender and relatedness. To assess the influence of various environmental factors on PTH, nonparametric tests were used due to non-normal distribution of PTH levels.

Results: We identified two potentially associated loci: rs28524851 on chromosome 22 in FAM19A5 gene ( $p=5.06*10^{-7}$ ,  $\beta=-0.33$ ,  $SE=0.06$ ) and rs6688219 on chromosome 1 in CASZ1 gene ( $p=7.96*10^{-7}$ ,  $\beta=-0.94$ ,  $SE=0.19$ ). Among females, PTH showed positive association with BMI and systolic blood pressure while smoking and alcohol consumption were negatively associated. Diastolic blood pressure was positively associated with PTH among males. Serum calcium levels and bone density showed negative association with PTH.

Conclusions: We found two potentially associated loci that need to be confirmed in a replication study. One of those is located in CASZ1 gene which is known to be associated with cardiac phenotypes. These results also indicate that environmental factors have influence on parathyroid function.

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## P18.087

### NMR metabolites association analysis to determine parent of origin effects

N. Pervjakova<sup>1,2</sup>, T. Haller<sup>1</sup>, S. Kasela<sup>1,2</sup>, R. Mägi<sup>1</sup>;

<sup>1</sup>Estonian Genome Center University of Tartu, Tartu, Estonia, <sup>2</sup>Department of Biotechnology, Institute of Molecular and Cell Biology, Tartu, Estonia.

Parent-of-origin effect (POE) occurs when the allelic effect on phenotype depends on whether the allele is inherited from mother or father. POE can be mediated through several mechanisms, such as genomic imprinting, transgenerational effects, in utero effects and the maternal environment. The aim of this study was to identify POE of common variants in NMR metabolites. We considered 5,853 individuals from Estonian Biobank (EGCUT) for 82 NMR metabolites. Individuals were imputed to the 1000 Genomes Project reference panel (March 2012 release). POE analysis was performed using POE method in QUICKTEST using markers with MAF  $\geq 1\%$ , info  $\geq 0.8$ . We observed evidence of POE for 7 metabolites ( $p < 5 \times 10^{-8}$ ) and are replicating these findings in an independent dataset. Identified variants are associated with lipids and lipoproteins.

Two of the found variants (rs1330350; rs189194743) located in TNC and PTPRD genes, which are associated with signaling molecules regulating a variety of cellular processes, such as neuronal regeneration, mitotic cycle, cell growth, neurons and axons migration during development. These regulatory features provide normal embryonic growth and therefore it suggests that identified POE may be mediated through genomic imprinting. This idea motivated us to perform further steps, such as allele-specific expression analysis, epigenome-wide association analysis with further replication in an independent cohort and methylation QTL to investigate mechanisms underlying POE and its association with genomic imprinting. We believe our results will contribute to uncovering complex relations between genetic variants and common traits and help to reveal some of the hidden heritability. Grants: EU30020; EU48695; SP1GVARENG.

## P18.088

### Individual-level pathway polygenic score method for identifying heterogeneous genetic bases of complex diseases

Y. Ruan, G. Breen, P.F. O'Reilly;

MRC Social, Genetic and Developmental Psychiatry Centre, IoPPN, King's College London, London, United Kingdom.

GWAS-based pathway analyses aim to identify biological pathways involved in the pathogenetic mechanisms of complex diseases. So far, these methods have generally focused on summary statistic results and identified the pathways that have causal effects at the whole sample level. Although existing approaches can highlight the involvement of causal pathways, they could not reveal the heterogeneous pathogenetic mechanisms across cases for a disease. Here, we develop a new method that utilizes individual-level genotype data to test for heterogeneity in the enrichment of risk alleles across different pathways for each individual - it does this by calculating polygenic risk scores specific to different pathways for each case individual. Our method aims to identify heterogeneity in the genetic basis of complex diseases, as well as potentially increase the overall power to identify causal pathways. Here we test this method on simulated case-control data in which the cases have heterogeneous causal pathways to assess its power to detect heterogeneity in pathway aetiology, and also compare its power for

overall pathway detection with existing summary statistic based methods. We exemplify the performance of our method to identify heterogeneity in pathways for several complex diseases, including schizophrenia and BMI, and compare our results with those from several well-established pathway analysis methods.

## P18.089

### Demographic inference of the Lithuanian population

A. Urnišytė, A. Molytė, E. Pranckevičienė, V. Kučinskas;

Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

The effective population size ( $Ne$ ) is one of the most important natural population parameters providing an insight into ancient structures of modern human populations. We estimated  $Ne$  and divergence time by analysing patterns of Linkage disequilibrium between autosomal single nucleotide polymorphisms (SNPs). We aimed to reconstruct past events of separation between Lithuanian ethnolinguistic groups Aukštaičiai and Žemaičiai, since these groups over a long time period might have developed as two independent Baltic tribes.

We used Illumina 770K *HumanOmniExpress-12v1.0* array data of 295 unrelated individuals of six ethnolinguistic groups of Lithuanian population (South Aukštaičiai (SA), East Aukštaičiai (EA), West Aukštaičiai (WA), South Žemaičiai (SŽ), West Žemaičiai (WŽ), North Žemaičiai (NŽ)). *NeON* R package was used to estimate  $Ne$  and divergence time.

Estimated  $Ne$  ranged from 4940 in the WŽ group to 5314 in the WA group. According to divergence time, the WŽ was earliest diverging group. The longest divergence time was observed between the WŽ and SA (9950 ya), and between the WŽ and EA (9650 ya), whereas the most recent separation occurred between the NŽ and SŽ groups (4775 ya).

It is ascertained that the overall effective population size of Žemaičiai is less in comparison with the overall of Aukštaičiai. More recent separations happened in ethnolinguistic groups residing in the same geographical area.

Our results support the hypothesis that Lithuanians originated from two Baltic ethnicities - Western Žemaičiai and South Aukštaičiai.

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## P18.090

### Investigating probable Turkish ancestry of Hungarians and Romani people on a genome-wide basis

Z. Bánfal<sup>1,2</sup>, A. Szabó<sup>1,2</sup>, B. L. Bálint<sup>3</sup>, B. I. Melegh<sup>1,2</sup>, K. Hadzsiev<sup>1,2</sup>, B. Melegh<sup>1,2</sup>,

<sup>1</sup>University of Pécs, Department of Medical Genetics, Pécs, Hungary, <sup>2</sup>University of Pécs, Szentágóthai Research Centre, Pécs, Hungary, <sup>3</sup>University of Debrecen, Department of Biochemistry and Molecular Biology, Debrecen, Hungary.

History of Hungarians, Roma and Turkic groups could have overlapped several times during their demographic activities. Ancestors of Hungarians, migrating from the Ural region to the Carpathian basin, were in contact with nomadic Turkic tribes. It is also known that significant part of recent Hungary fell to Ottoman control between the 16th Century for 150 years. Migration of Roma included also the Anatolian Island. Roma arrived to Hungary in the 15th century, fleeing from the conquering campaigns of the Ottoman Empire. Roma were a notable ethnic group of the Kingdom of Hungary in the 16th Century already.

According to these historical events, relationship of Hungarians and Roma with Turks was investigated based on genome-wide single nucleotide polymorphism (SNP) data. Clustering software, utilizing algorithmic and model-based approaches, formal tests of admixture, and identity-by-descent (IBD) segment analyzes were carried out attempting to shed light to the conclusion of these demographic events in a genome-wide basis. We attempted also to measure the impact of the Ottoman rule in East-Central Europe.

We confirmed that admixture of Roma and Turks during the Ottoman rule is also significant, and our estimation shows a proportion of 66% of total Turkic ancestry in Roma, compared to their Northwestern Indian ancestry. We estimate that Hungarians have an average IBD share length of 0.69Mb with Turks, which is significant, compared to other populations under Ottoman rule.

Our analyses confirmed that Ottoman occupation left detectable impact in East-Central Europe including Hungarians, and also shaped the ancestry of the Romani people.

**P18.091****Nationwide genomic study in Denmark reveals remarkable population homogeneity**

**G. Athanasiadis**<sup>1</sup>, **J. Cheng**<sup>1</sup>, **B. J. Vilhjálmsdóttir**<sup>1</sup>, **F. G. Jørgensen**<sup>2</sup>, **T. D. Als**<sup>1</sup>, **S. Le Hellard**<sup>3</sup>, **T. Espeseth**<sup>4</sup>, **P. F. Sullivan**<sup>5</sup>, **C. M. Hultman**<sup>5</sup>, **P. C. Kjærgaard**<sup>1</sup>, **M. H. Schierup**<sup>1</sup>, **T. Mailund**<sup>1</sup>, <sup>1</sup>*Aarhus University, Aarhus, Denmark*, <sup>2</sup>*Tørring Gymnasium, Aarhus, Denmark*, <sup>3</sup>*University of Bergen, Bergen, Norway*, <sup>4</sup>*University of Oslo, Oslo, Norway*, <sup>5</sup>*Karolinska Institutet, Stockholm, Sweden*

Denmark's genetic history has never been studied in detail. In this work, we analysed genetic and anthropometrical data from ~800 Danish students as part of an outreach activity promoting genomic literacy in secondary education. DNA analysis revealed remarkable homogeneity of the Danish population after discounting contributions from recent immigration. This homogeneity was reflected in PCA and AMOVA, but also in more sophisticated LD-based methods for estimating admixture. Notwithstanding Denmark's homogeneity, we observed a clear signal of Polish admixture in the East of the country, coinciding with historical Polish settlements in the region before the Middle Ages. In addition, Denmark has a substantially smaller effective population size compared to Sweden and Norway, possibly reflecting further lack of strong population structure. None of these three Scandinavian countries seems to have suffered a depression due to the Black Death in the Middle Ages. Finally, we used the students' genetic data to predict their adult height after training a novel prediction algorithm on public summary statistics from large GWAS. We validated our prediction using the students' self-reported height and found that we could predict height with a remarkable ~64% accuracy.

**P18.092****Fine-scale population structure in Western France: Loire River as genetic barrier**

**J. Giemza**<sup>1</sup>, **F. Simonet**<sup>1,2</sup>, **M. Karakachoff**<sup>1,2</sup>, **K. Rouault**<sup>3</sup>, **E. Charpentier**<sup>4</sup>, **S. Lecointe**<sup>1,2</sup>, **P. Lindenbaum**<sup>1,2</sup>, **J. Violleau**<sup>1,2</sup>, **C. Férec**<sup>5</sup>, **H. Le Marec**<sup>1,2</sup>, **S. Chatel**<sup>1,2</sup>, **J. Schott**<sup>1,2</sup>, **E. Génin**<sup>2</sup>, **R. Redon**<sup>1,2</sup>, **C. Dina**<sup>1,2</sup>;

<sup>1</sup>*Inserm UMR 1087 ; CNRS UMR 6291 ; Université de Nantes ; l'institut du thorax, Nantes, France*, <sup>2</sup>*CHU Nantes, l'institut du thorax, Nantes, France*, <sup>3</sup>*Inserm UMR 1078, CHRU Brest, University Bretagne Occidentale, EFS, Brest, France*

**Background:** The genetic structure of human populations varies throughout the world, being influenced by migration, admixture, natural selection and genetic drift. Human population structure has first been investigated at broad scales, between and within continents. Currently researchers focus on finer scales, examining genetic structure within countries or regions.

**Characterising such genetic variation is of interest as it provides insight into demographical history and informs research on disease association studies, especially the ones focusing on rare variants.**

**Results:** We genotyped 456 individuals from Western France Atlantic Coast, from Finistère to Vendée, with at least three of their grandparents born within a 15 kilometres distance using Axiom CEU Chip. Principal Components analysis revealed that individuals from the same departments form clusters and we observed a high correlation between geographical position and components (p-value < 2e-16). The main geographical barrier in the region is Loire River. Many independent methods support the hypothesis that Loire River is also a genetic barrier. The two groups of individuals, from north or south of Loire, are well differentiated along PC1 axis. ADMIXTURE estimated different ancestry proportions for the two groups. The first split of hierarchical clustering returned by fineSTRUCTURE, and the one based on normalized counts of identity-by-descent segments is between north and south of Loire.

**Conclusion:** We here report both evidence for isolation by distance and existence of a genetic barrier, the Loire River. The discovered fine-scale population structure may have consequences in association analyses, especially for rare variants which tend to be geographically clustered.

**P18.093****Association analysis between quantitative traits and genetic marker for longitudinal data**

**M. Lin**<sup>1</sup>, **J. Chien**<sup>1</sup>, **S. Lin**<sup>1</sup>, **W. Y. Wu**<sup>1</sup>, **L. Chen**<sup>2</sup>, **A. M. Yen**<sup>3</sup>, **T. Liu**<sup>4</sup>, **H. Chen**<sup>5</sup>;

<sup>1</sup>*Institute of Public Health, National Yang-Ming University, Taipei, Taiwan*, <sup>2</sup>*School of Oral Hygiene, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan*, <sup>3</sup>*School of Oral Hygiene, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan*, <sup>4</sup>*Lienchiang County Government, Matsu, Lienchiang County, Taiwan*, <sup>5</sup>*Institute of Epidemiology and Preventative Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan*

**Introduction:** Quantitative traits are generally changed with time. Longitudinal genetic studies with multiple measurements offer a valuable resource to examine how genetic and environmental factors that may affect complex

traits over time. However, most of genetic studies of quantitative traits using longitudinal data did not take into account genetic effects that may interact with age. Fan et al. (2012) develop penalized spline-based mixed model to estimate the temporal trend of mean effects and genetic effects. Nevertheless, their simulation study did not consider gene-time interaction.

**Methods:** We conducted a simulation study to investigate the performance of spline-based mixed model and three parametric mixed models on the settings of gene-time interactive effects as well as gene-time invariant effects. This spline-based mixed model was also applied to analyze the association between the Matsu Community-Based Integrated Screening (MA-CIS) program longitudinal blood pressure data and six single nucleotide polymorphisms in the *KLOTHO* gene.

**Results:** Our simulation study showed that spline-based mixed model had better power and less bias square than other parametric mixed models for both gene-time invariant effect and gene-time interactive effect. For the MA-CIS data, we found the haplotype GGACTA was associated with blood pressure and the SNP rs1207568 and haplotype AGCCA had gene-time interactive effects with blood pressures. **Conclusion:** Our study demonstrated that the spline-based mixed model is more flexible than the parametric mixed models as it could conveniently capture the pattern of relationship between quantitative traits and genetic markers.

**P18.094****Boosting the power of the sequence kernel association test (SKAT) by properly estimating its null distribution**

**K. Wang**;

*University of Iowa, Iowa City, IA, United States*

The sequence kernel association test (SKAT) is probably the most popular statistical test used in rare-variant association studies. Its null distribution involves unknown parameters that need to be estimated. The current estimation method has valid type I error rate but the power is compromised as all subjects are used for estimation. We develop a novel estimation method that uses only „control“ subjects. Named SKAT+, this new method uses the same test statistic as SKAT but differs in the way the null distribution is estimated. It is more powerful than SKAT almost surely as sample size increases. Extensive simulation studies and an application to a Genetic Analysis Workshop 17 data demonstrate its overall superior power performance over SKAT while maintain control over type I error rate. The proposed approach is easily applicable to existing numerous variations of SKAT in the literature.

**P18.095****Heritability and genome-wide association analyses of sleep duration in children: the EAGLE consortium**

**M. Bustamante**<sup>1</sup>, **M. Marinelli**<sup>2</sup>, **I. Pappa**<sup>3</sup>, **C. Bonilla**<sup>4</sup>, **A. Suarez**<sup>5</sup>, **C. M. Tiesler**<sup>6</sup>, **N. Vilor-Tejedor**<sup>1</sup>, **H. M. Zafarmand**<sup>7</sup>, **M. Alvarez-Pedrerol**<sup>1</sup>, **S. Andersson**<sup>8</sup>, **M. J. Bakermans-Kranenburg**<sup>9</sup>, **X. Estivill**<sup>10</sup>, **D. M. Evans**<sup>11</sup>, **C. Flexeder**<sup>9</sup>, **J. Forns**<sup>1</sup>, **J. R. Gonzalez**<sup>1</sup>, **M. Guxens**<sup>1</sup>, **A. Huss**<sup>11</sup>, **M. H. van IJzendoorn**<sup>3</sup>, **V. W. V. Jaddoe**<sup>12</sup>, **J. Julvez**<sup>1</sup>, **J. Lahti**<sup>13</sup>, **M. López-Vicente**<sup>1</sup>, **M. J. López-Espinosa**<sup>13</sup>, **J. Manz**<sup>14</sup>, **V. R. Mileva-Seitz**<sup>15</sup>, **M. Perola**<sup>16</sup>, **A. Pesonen**<sup>15</sup>, **F. Rivadeneira**<sup>12</sup>, **P. P. Salo**<sup>17</sup>, **S. Shahand**<sup>18</sup>, **H. Schulz**<sup>6</sup>, **M. Standl**<sup>6</sup>, **E. Thiering**<sup>6</sup>, **N. J. Timpons**<sup>4</sup>, **M. Torrent**<sup>19</sup>, **A. Uitterlinden**<sup>12</sup>, **G. Davey Smith**<sup>4</sup>, **M. Estarlich**<sup>13</sup>, **J. Heinrich**<sup>6</sup>, **K. Räikkönen**<sup>5</sup>, **T. G. M. Vrijkotte**<sup>20</sup>, **H. Tiemeier**<sup>12</sup>, **J. Sunyer**<sup>4</sup>;

<sup>1</sup>*ISGlobal, CREAL, Barcelona, Spain*, <sup>2</sup>*AQUAS, Barcelona, Spain*, <sup>3</sup>*School of Pedagogical and Educational Sciences, Erasmus University Rotterdam, Rotterdam, Netherlands*, <sup>4</sup>*School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom*, <sup>5</sup>*Institute of behavioural sciences, University of Helsinki, Helsinki, Finland*, <sup>6</sup>*Institute of Epidemiology I, Helmholtz ZentrumMünchen - German Research Center for Environmental Health, Neuherberg, Germany*, <sup>7</sup>*Department of Public Health, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands*, <sup>8</sup>*Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland*, <sup>9</sup>*Centre for Child and Family Studies, Leiden University, Leiden, Netherlands*, <sup>10</sup>*CRG, Barcelona, Spain*, <sup>11</sup>*Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands*, <sup>12</sup>*Generation R Study Group, Erasmus Medical Center, Rotterdam, Netherlands*, <sup>13</sup>*FISABIO - Universitat Jaume I - Universitat de València, Valencia, Spain*, <sup>14</sup>*Research Unit of Molecular Epidemiology, Helmholtz ZentrumMünchen, German Research Center for Environmental Health, Neuherberg, Germany*, <sup>15</sup>*Centre for Child and Family Studies, Leiden University, Leiden, Spain*, <sup>16</sup>*Public Health Genomics Unit and Institute for Molecular Medicine, University of Helsinki, Helsinki, Finland*, <sup>17</sup>*Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland*, <sup>18</sup>*Department of Clinical Epidemiology Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands*, <sup>19</sup>*Ib-salut Area de Salut de Menorca, Menorca, Spain*

**Introduction:** Low or excessive sleep duration has been associated with multiple health outcomes, however the biology behind these associations remains elusive. Specifically, genetic studies in children are scarce. In this study, we aimed to: (1) estimate the proportion of genetic variance of sleep duration in children attributed to single nucleotide polymorphisms (SNPs),

(2) identify novel SNPs associated with sleep duration, and (3) investigate the genetic overlap of sleep duration and metabolic and psychiatric outcomes.

**Material and methods:** 10,554 European children aged 2-11y from cohorts participating in the EARly Genetics and Life course Epidemiology (EAGLE) Consortium were included in the study. Sleep duration was assessed through questionnaires. Fixed-effects meta-analyses were performed on 1000G imputed GWAS data using METAL. Heritability was estimated with GCTA and LD score regression. LD score regression was also used to test genetic correlations.

**Results:** We found evidence of significant, but modest SNP heritability of sleep duration in children (SNP  $h^2$  0.14, 95% CI 0.05, 0.23). A novel region on chromosome 11q13.4 (rs74506765, minor allele frequency=0.1,  $p=2.27e-08$ ) was associated with sleep duration, but it was not replicated in an independent sample of children (N= 1,250). Significant genetic overlap ( $r^2= .23$ ,  $p=.05$ ) between sleep duration in children and type 2 diabetes (T2D) in adults was detected, while no correlation was observed with other metabolic or psychiatric outcomes.

**Conclusions:** The significant SNP heritability of sleep duration in children, and the genetic overlap with T2D support the continuation of studies investigating the genetic mechanisms linking sleep duration to health outcomes.

## P18.096

### Devising a SNP-based panel for human identification in Indian populations

*A. Sarkar, M. R. Nandineni;*

*Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India.*

**Background and aim:** Currently, forensic DNA profiling comprise of amplifying a battery of 14-16 short tandem repeat (STR) loci which often fails when the DNA is highly degraded. Single nucleotide polymorphism (SNP) has been proposed and studied as an alternative for human identification (HID), but for Indian populations, SNP-based panel has not been explored. The current study aims towards building a SNP-based panel for Indian populations for forensic HID.

**Methods:** A bioinformatic approach was utilized to shortlist SNPs from public databases which were subsequently genotyped from the salivary DNA of ~460 unrelated adult volunteers in Indian populations using *GoldenGate®* Genotyping Assay (Illumina, Inc, USA). Based upon the distribution of the alleles in the tested populations (grouped as North, East, West and South India) the SNPs with most desired characteristics were accepted.

**Results:** Screening public databases from worldwide populations led us to shortlist a panel of 270 SNPs which were genotyped in Indian populations and finally, 2-4 SNPs from each autosome were selected to devise a panel of 70 SNPs. Random match probability (RMP) of the panel was  $\sim 10^{-29}$  for all the regions which was higher than panels currently available for forensic DNA profiling.

**Discussion and conclusion:** Considering several factors, a SNP-based panel for India has been devised which is highly efficient, is expected to ensure conclusive results even in challenging forensic samples and can be considered for other world populations as well.

The study was funded from the core grant of Centre for DNA Fingerprinting and Diagnostics (CDFD).

## P18.097

### A population-level analysis of mutations affecting splicing

*P. Puigdevall, R. Castelo;*

*Universitat Pompeu Fabra, Barcelona, Spain.*

Wild-type processing of RNA transcripts by the splicing machinery is a fundamental step in the gene expression pathway. Mutations affecting this step can produce aberrant splicing with deleterious effects that ultimately lead to disease. Mutation databases curating the scientific literature store an increasing number of single nucleotide variants (SNVs) inducing aberrant splicing involved in disease. Yet, this increase is far below the current growth rate of genetically profiled diseased individuals. This results in many SNVs of unknown effect. In this context, understanding the deleterious effects of SNVs on splicing becomes extremely important to attempt a sensible prioritization of such SNVs. Population-level allele frequencies constitute a valuable resource to gather understanding of mutation processes. We have used the last release of the 1000 Genomes and the ExAC catalogs of human variation to characterize mutations that affect splicing and attempt a sensible prioritization of such SNVs.

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## P18.098

### High-order epistasis explains majority of still-missing heritability for human height and BMI

*R. de Vlaming, C. A. Rietveld;*

*Erasmus University Rotterdam Institute for Behavior and Biology, Rotterdam, Netherlands.*

Narrow-sense heritability ( $h^2$ ) estimates for complex traits based on common SNPs are generally substantially lower than estimates obtained from the classical twin-design, a disparity referred to as the 'still-missing heritability'. By estimating the variance components of a SNP-based ACE model in a sample of monozygotic and dizygotic twins, we show that imperfect tagging of genetic variation by common SNPs does not fully account for the large difference between SNP-based and classical twin-study  $h^2$  estimates for human height and BMI. However, a SNP-based model that accounts for high-order epistatic effects provides  $h^2$  estimates for human height and BMI that agree with previously reported SNP-based estimates; 47% for height (SE=4%) and 28% for BMI (SE=5%). Moreover, the difference between  $h^2$  estimates from the epistatic model and the classical twin-design coincide with the theoretically expected bias in twin-study estimates under a high-order epistatic model. Given that the twin-study assumption of equal environment for monozygotic and dizygotic twins holds, these results provide evidence that twin study  $h^2$  estimates are biased upwards due to the misattribution of epistatic effects to additive effects in the classical ACE model. The bias due to epistasis is able to explain the majority of the still-missing heritability for human height and BMI.

## P18.099

### Increasing power to infer relatedness and ancestry by using haplotype information

*G. Hellenthal;*

*University College London, London, United Kingdom.*

Understanding and correctly modelling the genetic structure of individuals sampled world-wide is essential for both designing and interpreting genotype-phenotype association studies. As both the number of individuals and number of genetic markers increase with the onslaught of new e.g. large-scale sequencing studies, more subtle -- and likely regional -- genetic variation patterns will be captured, enabling the elucidation of (and necessitating accounting for) genetic differences at increasingly finer geographic scales. I demonstrate how using haplotype information, which exploits correlation patterns among neighbouring Single-Nucleotide-Polymorphisms (SNPs) or other variants, increases precision to infer genetic relatedness and ancestry over the far more commonly-used programs that ignore this information. In particular I highlight how incorporating haplotype information identifies genetic patterns at fine geographic scales, e.g. within a country, where standard approaches fail. I describe new statistical methodology that infers the ancestry and relatedness of individuals by comparing haplotype patterns among an arbitrarily large number of world-wide groups, while accounting for sampling artefacts such as unequal group sample sizes and readily accommodating DNA samples from ancient human remains. I apply this model to individuals from geographically localized regions of Africa and Europe to elucidate population sub-structure, infer haplotype sharing patterns among groups, discover and date past interbreeding events (i.e. admixture) and identify regions of the genome affected by selection events.

## P18.100

### Identification of genetic markers associated with high density lipoprotein cholesterol by genome-wide screening in Tehran cardio-metabolic genetic study (TCGS)

*M. S. Daneshpour<sup>1</sup>, M. Fallah<sup>1</sup>, B. Sedaghatkhayat<sup>1</sup>, K. Guity<sup>1</sup>, F. Azizi<sup>2</sup>;*

*<sup>1</sup>Cellular and Molecular Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of,*

*<sup>2</sup>Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.*

**Introduction:** Genome-wide association studies (GWAS) have become an important strategy for genetic examination of human complex diseases. Present study is the first GWAS in Middle East and tried to investigate and replicate the association between HDL-C concentration and candidate loci in Tehran cardio-metabolic genetic (TCGS) participants.

**Materials and methods:** In TCGS, 8296 subjects analyzed for 623393 quality-checked SNPs (HumanOmniExpress-24-v1-0 bead at deCODE genetics). Serum HDL level was used as the main phenotype indicator with normal distribution and outlier data ( $\pm 4SD$ ) were excluded. Efficient Bayesian mixed-model analysis with the help of Bolt-LMM v2.1 was used for association analysis in total population, male and female separately.

Results: At the genome-wide level ( $p < 10^{-7}$ ), 45 SNPs were statistically associated with circulating HDL-cholesterol concentrations in total population that distributed in 8p21.3, 8p23.1, 11q23.3, 15q21.3, 16q13 genomic regions. Most of these SNPs were confirmed in male and female separately.

Conclusion: In this study we discovered 5 loci with 6 protein coding gene (ALDH1A2, APOA5, CETP, LPL, PIEZO2, SLC12A3); ncRNA (LOC157273); Novel miRNA (AC012181.1) related to HDL variation. We provide genetic analysis in relation to HDL in Iranian population with high prevalence of low HDL. This study is the first GWAS in Iran and Understanding the molecular, cellular and clinical consequences of the newly identified loci may inform therapy and clinical care.

#### P18.103

#### Genome-wide survival analysis of skin cancer in a Celtic renal transplant population

C. P. Stapleton<sup>1</sup>, P. B. Mark<sup>2</sup>, P. J. Phelan<sup>3</sup>, P. J. Conlon<sup>4</sup>, G. L. Cavalleri<sup>1</sup>;

<sup>1</sup>Department of Molecular and Cellular Therapeutics, Royal College of Surgeons Ireland, Dublin, Ireland, <sup>2</sup>Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom, <sup>3</sup>Department of Nephrology, Royal Infirmary of Edinburgh, NHS Lothian, United Kingdom, <sup>4</sup>Beaumont Hospital Kidney Centre, Dublin, Ireland.

Renal-transplant recipients have a 33-fold increased risk of developing non-melanoma skin cancer relative to age-matched non-transplanted individuals. Some of this risk can be attributed to factors including type of immunosuppressant treatment, however much of the risk remains unaccounted for. A mega-analysis of dense gene-chip data was conducted between datasets from Dublin and Glasgow to map germline genetic variations influencing time to onset of skin cancer post-transplantation.

Genotyping was carried out using the Illumina Human610-Quad BeadChip. Genome-wide survival analysis was conducted using a Cox proportional hazards model. Recipient age, recipient gender and the first 4 principal components were used as covariates. A genome-wide significance threshold of  $5 \times 10^{-8}$  was used.

In the Dublin dataset (n cases=94; n controls=231), a significant novel association ( $p=4 \times 10^{-8}$ ) was found between time to developing skin cancer post-transplantation and the variant, rs12519378, in SPOCK1. Heterozygote individuals developed skin cancer 7 times faster than wild-type homozygotes. The significance ( $p=2 \times 10^{-5}$ ) of rs12519378 fell in the mega-analysis when the Glasgow cohort (n cases=5; n controls=64) was added. No other SNPs achieved the threshold of significance.

SPOCK1 expression activates the PI3K/AKT pathway thus inhibiting apoptosis and promoting oncogenesis. The results presented here hint at a potential role of SPOCK1 in the development of post-transplant skin cancer. However, further data is required to determine the robustness of the SPOCK1 signal and identify other signals of association. We are currently seeking to extend this analysis to additional patient cohorts.

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#### P18.105

#### Stop variants identified by exome-sequencing in patients and controls for Type 2 Diabetes Mellitus (T2DM) from Spanish population

V. Lendínez-Tortajada<sup>1</sup>, I. Galán-Chilet<sup>1</sup>, D. Pérez-Gil<sup>1</sup>, A. Sanchis-Juan<sup>1</sup>, P. Marín-García<sup>1</sup>, A. B. García-García<sup>1,2</sup>, F. J. Chaves<sup>1,2</sup>;

<sup>1</sup>Genotyping and Genetic Diagnosis Unit, Biomedical Research Institute-INCLIVA, Valencia, Spain, <sup>2</sup>CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), Barcelona, Spain.

Introduction: Type 2 Diabetes (T2DM) explains the 90-95% of diagnosed cases with Diabetes Mellitus. It is a multifactorial disease affected by ambient and genetic factors. The genetic component appears to be very high and T2DM inheritability is around the 70%. Up to now, the studies have only proved the 10-15% of this component. The aim of our study is the identification of rare variants that can explain a part of genetic component of T2DM. Material and Methods: We performed a study in the Spanish population, of 200 healthy controls and 200 patients with T2DM from 40-65 years, sequencing their exome with the System HiScanSQ of Illumina®. By bioinformatics data analyses, we identified rare variants with theoretically functional impact on the phenotype, detected in cases or controls but do not found in both. From these variants we selected the gained/loss stop mutations and were verified by Sanger sequencing.

Results: We have identified 102 gained/loss stop variants in the control group and 50 gained/loss stop variants in the diabetic group.

Conclusion: We have described new variants with a possible related functional effect with diabetes and protection against its development; some of them are in genes not previously associated with the disease. Further studies are needed in order to establish specific associations between variants and T2DM.

### P19 Genetic counselling/Education/public services

#### P19.01

#### Actionable mutations in the whole-genome sequenced gene donors of Estonian Genome Center

N. Tönnisson<sup>1,2</sup>, T. Nikopensius<sup>1</sup>, K. Vaikull<sup>1</sup>, L. Leitsalu<sup>1</sup>, M. Kals<sup>1</sup>, P. Padrik<sup>3</sup>, T. Esko<sup>1,4</sup>, A. Metspalu<sup>1,5</sup>;

<sup>1</sup>Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>2</sup>Dept. of Genetics, Tartu University Hospital, Tartu, Estonia, <sup>3</sup>Oncology Clinic, Tartu University Hospital, Tartu, Estonia, <sup>4</sup>Broad Institute, Cambridge, MA, United States, <sup>5</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Introduction: In 2013, American College of Medical Geneticists (ACMG) has issued a minimal list of 24 actionable conditions and 56 related genes of medical utility if incidentally found mutated in clinical whole genome and whole exome sequencing. The data were suggested to be reported back to sequenced individuals irrespective of their gender and age.

Aims, materials and methods: In Estonian Genome Center, we have performed whole genome sequencing of over 2200 individuals selected to be a representative subset of Estonian population with approximately 30x mean coverage. The data is meant to be used for a variety of further population and health-related studies. As one of the aims of our biobank is to provide feedback to the gene donors related to their health risks, we were searching the sequencing data for known and expected pathogenic mutations, starting from the ACMG gene list as the first priority.

Results: Based on initial filtering, database and literature search, we were able to detect 33 variants in 20 genes related to 13 clinical conditions in the ACMG list mentioned above. 45 individuals (2% of the sequenced cohort) carry the variants. One of the surprises was that 16 individuals with known and expected BRCA1 and BRCA2 pathogenic mutations (0.7% of total) were detected in the sequencing cohort.

Perspectives: The sequencing data will be further validated and used as to set up a health-related feedback routine in our biobank, together with the required professional counselling. The confirmed cases will get any further required specialist medical support.

#### P19.02

#### Towards and beyond the Eureka: The role of disease advocacy groups in diagnostic odyssey and benefits of collaborating with them more frequently

V. Okur<sup>1</sup>, M. E. Schwartz<sup>2</sup>, F. J. D. Smith<sup>2</sup>;

<sup>1</sup>Haydarpaşa Numune Research & Education Hospital, Istanbul, Turkey, <sup>2</sup>Pachyonychia Congenita Project, Salt Lake City, UT, United States.

Background: Diagnosing a rare disease is very important for geneticists so much so that we can describe it as eureka moment for us. Correspondingly, geneticists usually know the general rare disease statistics such as total number of rare diseases and percentage of affected population worldwide very well. Unfortunately, most of us are not aware of how many rare disease advocacy groups there are, what their struggles are and how they can also change the patients' journey towards diagnosis.

Patients: Two first cousins with congenital nail dystrophies were referred with a differential diagnosis of pachyonychia congenita. Due to technical and economic limitations we contacted disease advocacy group, Pachyonychia Congenita Project, to seek help for molecular studies. After reviewing the cases the genetics team associated with the advocacy group proposed to screen another recently defined rare entity, "autosomal recessive nail dysplasia", and found a new mutation in a causative gene, FZD6, within 2 months.

Discussion: The role of patient advocacy groups in human genetics is undeniable. Apart from providing support for patients, their determination may have saved 5-10 years in treatment discoveries of some rare diseases. Nowadays, with the advance of genetic technologies we often forget them except for some devoted physicians and scientists. However, patient advocacy groups are experts in their rare disorder and can play a crucial role, as in our cases in determining a correct diagnosis and preventing unnecessary tests and/or treatment. Therefore, it can be beneficial to consult with disease advocacy groups at early stages of diagnostic odysseys.

## P19.03

### Array Comparative Genomic Hybridisation result communication: navigating uncertainty in the Genetics Clinic.

*S. Aziz<sup>1</sup>, L. Bennett<sup>1</sup>, S. Hewitt<sup>2</sup>, M. Ahmed<sup>2</sup>, K. Prescott<sup>3</sup>, \*Contributed equally; <sup>1</sup>School of Medicine, University of Leeds, Leeds, United Kingdom, <sup>2</sup>Leeds Genetics Laboratory, Ashley Wing, St James's Hospital, Leeds, United Kingdom, <sup>3</sup>Yorkshire Regional Genetics Service, Clinical Genetics Department, Chapel Allerton Hospital, Leeds, United Kingdom.*

**Introduction:** Array Comparative Genomic Hybridisation (aCGH) allows whole genome analysis at a higher resolution than was previously possible by karyotyping alone. This has resulted in the increasing detection of ambiguous results, termed 'Variants of Uncertain Significance' (VOUS). VOUS results pose communication challenges to clinicians and families. Currently there are no universal guidelines for aCGH result communication. This study evaluates the counselling practice of aCGH results of differing pathogenicities.

**Methods:** This observational study included 75 paediatric patients who had aCGH testing at the Yorkshire Regional Genetics Service, UK. Cases were randomly selected from three result categories; Clinically Significant (n=24), VOUS Likely Benign (n=25) and VOUS Uncertain (n=26). Data regarding the communication of results was collected from patient records. Descriptive statistics and Fisher's Exact test were applied to the data.

**Results:** Pre-test counselling regarding the possibility of a VOUS result was documented in 5.3% of cases. There was a significant difference in the mode of result disclosure between categories ( $p=0.001$ ). 75.0% of Clinically Significant results were disclosed via face-to-face consultation and letter, whereas 74.5% of VOUS results were disclosed via letter only. 23.5% of those receiving VOUS results were advised to re-contact genetics services in the future.

**Conclusions:** Communication of aCGH results varies according to their pathogenicity. To standardise practice, all patients should be counselled about the possibility of a VOUS result. The effect of mode of disclosure on result comprehension should be studied further. Patients receiving VOUS results should be advised to re-contact genetics services to seek new information.

## P19.04

### The evolution of an annual information model for BRCA1/2 gene fault carriers

*A. Wijesinghe, D. Petrie; Genetic Alliance Australia, Darlinghurst, Australia.*

**Introduction:** Individuals with BRCA1/2 gene faults are a unique group with specific support needs. Genetic Alliance Australia (GA), a peak umbrella organisation, was approached by a consumer and a genetic counsellor in 2001, seeking support for BRCA1/2 gene fault carriers. The outcome was an annual BRCA1/2 information day hosted by GA for 15 years, an alternative to traditional support group models. GA documented how this unique model evolved with patients' support needs over time.

**Method:** Qualitative and quantitative data was collected and analysed from completed evaluations over 15 years. Patients' perspectives and suggestions for improvements/topics for the day were recorded and logistical elements analysed.

**Results:** Evaluation results showed on average 73% of annual attendees were new. GA continually revaluated and modified the format based on feedback, catering to new and repeat attendees. Participants consistently responded positively to the diversity of information presented and appreciated opportunities to ask health professionals questions in a non-clinical setting. Participants appreciated hearing personal stories and embraced the opportunity to communicate and share their experiences with others who were at different points in their journey with BRCA1/2 gene faults. Life changing decisions and challenges were openly shared. Partnership with familial cancer genetic counsellors and consumers has grown from a consultative group into a cohesive and integrated partnership.

**Conclusion:** The BRCA1/2 Information Day provides a non-clinical, face-to-face approach focusing on mutual aid amongst participants. It has evolved into a unique hybrid of support group and information day models, driven by consumers' needs, demonstrating its portability.

## P19.05

### BRCA population screening in unaffected Ashkenazi Jewish women. A Randomized Controlled Trial of different pre-test strategies

*S. Lieberman<sup>1,2</sup>, A. Tomer<sup>1</sup>, A. Ben-Chetrit<sup>1,2</sup>, O. Olsha<sup>3</sup>, S. Levin<sup>1</sup>, R. Beer<sup>1</sup>, A. Raz<sup>5</sup>, A. Lahad<sup>4</sup>, E. Levy-Lahad<sup>1,2</sup>;*

<sup>1</sup>Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel, <sup>2</sup>Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel, <sup>3</sup>Surgical Department, Shaare Zedek Medical Center, Jerusalem, Israel, <sup>4</sup>Family Medicine, Clalit Health Services,

*Shoham, Israel, <sup>5</sup>Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>6</sup>Department of Family Medicine, Clalit Health Services, Jerusalem, Israel.*

Approximately half of BRCA1/BRCA2 carriers lack significant family history, and would only be identified through screening. Ashkenazi Jewish (AJ) population is a model for screening, given high prevalence (1/40) and testing sensitivity (>95%) of three common mutations. Towards implementation, we aim to examine the impact of excluding pre-test genetic counseling (GC) in the population screening setting.

Healthy AJ women age  $\geq$  25 years are randomized to two pre-test arms: written information only (WI) vs. GC. Post-testing, GC is provided to high-risk non-carriers and to all carriers. Psychosocial outcomes (satisfaction, stress, personal perceived control (PPC), knowledge) are assessed one week (Q1) and 6 months (Q2) post-testing.

Among the first 749 participants (mean age 46 years), we identified 11 carriers (1.5%). Only 3/11 carriers had significant family history. Post-testing, 95% of GC and 94% of WI participants report being satisfied with testing. Stress (IES) scores were similar in both groups. At Q1, PPC scores and knowledge were higher in GC ( $p=.005$ ;  $p=.0001$ ). At Q2, only PPC scores remained higher in GC: 1.39 vs. 1.25 in WI ( $p=.02$ ). Carriers had higher PPC and knowledge than non-carriers. At Q2, carriers' stress level was higher (14.9 vs. 5.3,  $p=.0006$ ), as expected.

Screening would identify substantially more carriers (regardless of family history). Compared to WI, pre-test GC provides a mild, temporary, increase in knowledge, accompanied by a greater sense of control. Forgoing pre-test GC may be an alternative for screening, particularly if alternative methods for imparting knowledge are explored.

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## P19.06

### Variant Reclassification in Cancer Genetic Testing: Are Genetic Counselors Prepared? A Review of Current Practices

*N. White<sup>1</sup>, B. White<sup>2</sup>, C. Radford<sup>3</sup>, K. Brooks<sup>4</sup>;*

<sup>1</sup>Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom, <sup>2</sup>Levine Cancer Institute, Carolinas Medical Centre, Charlotte, NC, United States, <sup>3</sup>Invitae, San Francisco, CA, United States, <sup>4</sup>South Carolina Oncology Associates, Columbia, SC, United States.

Cancer genetics is a critical specialty that is continuously advancing; genetic counsellors must be in sync with these advancements. Currently, multi-gene testing is being adapted into the genetic counselling setting. The 'hot topic' of an increased likelihood of variant results has been explored previously. However, research surrounding VUS reclassification is lacking. This study aimed to identify current practices of cancer genetic counsellors with regard to variant reclassification.

**Method:** This study utilised an online survey. SAS 9.4 was employed for quantitative data, qualitative data was analysed for themes.

**Results:** We determined that cancer genetic counsellors handle the reclassification of VUS results in a unified manner. 95% of respondents ( $n = 209$ ) discuss reclassification with their patients upon receiving a VUS result. Similarly, 95% of respondents ( $n = 209$ ) will sometimes or always make a plan to communicate VUS reclassifications should they arise in the future. The majority (97%) ( $n = 183$ ) indicated that the protocol for re-contacting patients with a VUS reclassification wouldn't be different from what they had used for single-gene analysis. Varying opinions existed on whether practice guidelines are necessary, a number expressed concerns surrounding liability issues and the feasibility of implementing recommendations across different institutions.

**Conclusions:** Study findings indicate most genetic counsellors are utilising unified practices when handling variant reclassifications. A proportion felt guidance for certain areas relating to variant reclassifications are necessary. Given the coming wave of reclassifications from multiple collaborative efforts to reclassify VUS results future research must explore in-depth the issues identified in this project.

## P19.07

### Improving referral criteria for cancer risk assessment from primary care centers and community hospitals in the era of new indications for genetic testing.

*A. Solanes<sup>1</sup>, E. Grau<sup>1</sup>, A. Velasco<sup>2</sup>, E. Darder<sup>2</sup>, A. López<sup>2</sup>, M. Salinas<sup>3</sup>, S. Iglesias<sup>3</sup>, Á. Teulé<sup>3</sup>, A. Izquierdo<sup>2</sup>, C. Lázaro<sup>3</sup>, G. Capella<sup>3</sup>, J. Brunet<sup>2</sup>, M. Navarro<sup>1</sup>;*

<sup>1</sup>Hereditary Cancer Program, Catalan Institute of Oncology, Badalona, Spain,

<sup>2</sup>Hereditary Cancer Program, Catalan Institute of Oncology, Girona, Spain, <sup>3</sup>Hereditary Cancer Program, Catalan Institute of Oncology, L'Hospitalet, Spain.

Identify the patient/families with inherited cancer (IC) improves the prevention, treatment and survival.

The increasing demand of genetic assessment collapsed our cancer genetic counselling unit (CGCU) in 2015. Covered by a genetic counsellor and an oncologist specialized in IC, we increased from 142 first visits in 2008 up to 570 on 2014. It became necessary to review the activities and resources to optimize it all. One of the improvements was to provide the professionals that collaborate with us with tools to filter the suitable cases. Goals: to confirm the attention of non appropriate cases in order to solve it, to explain last guides, to provide feedback between specialists and CGCU. In January 2015 we reviewed 99 first visits of 2014. We classified them on the risk level of IC, 47,5% of high risk, 25,25% of moderate risk and 27,25% of non risk. We wrote the project and didactic material. We contacted the contributor centers to set up the training sessions and did workshops. In January 2016, we reviewed the last 93 first visits and classified them too, 71% of high risk, 17,2% of moderate risk and 11,8% of non risk. (80w)

After the project, the patient referral criteria has improved 23%. The didactic material is available (intranet). There's a new contacts network, and a new online access to final report of CGCU.

We have to adapt ourselves to the increasing usage of cancer genetics with current resources. Our results confirm that increasing the knowledge improves the quality of the service.

share information online.

Using a cross-sectional survey design, we have collected data on patient preferences via an online questionnaire. We are recruiting patients via charity websites, genetics clinics and colorectal clinics. Quantitative data were analysed using descriptive statistics and qualitative data from free text responses were analysed for recurrent themes. Preliminary results indicate that most (47/60) would like information in other ways; via email, websites or social media, but also through follow-up appointments (33/55). Respondents wanted more information about genetic testing (31/59), healthy lifestyle (24/59) and talking to children (20/59). Initial data illustrate a diverse experience of families' access to appropriate advice and screening. There is an appetite for more comprehensive information and a recurring theme of "having to fight for screening all the time". Updated results will be given. Results indicate a pragmatic approach is needed to help relatives share information. Building on these data, telephone interviews of a purposive sample of respondents will guide development of a website. We propose to use and evaluate digital technology to enhance support to patients and facilitate information sharing within families. The results may be applicable for genetic conditions beyond cancer.

#### P19.08

#### Twenty years of external quality assessment for cystic fibrosis demonstrates marked improvement

L. Tembuyser<sup>1</sup>, M. Schwarz<sup>2</sup>, E. Girodon<sup>3</sup>, M. Morris<sup>4</sup>, M. Audrezet<sup>5</sup>, S. Berwouts<sup>6</sup>, C. Meyer-Kleine<sup>7</sup>, C. Raynal<sup>8</sup>, D. Radojkovic<sup>9</sup>, M. Stuhrmann<sup>10</sup>, H. Torkler<sup>11</sup>, R. Yamamoto<sup>12</sup>, E. Dequeker<sup>1</sup>,

<sup>1</sup>KU Leuven - University of Leuven, Leuven, Belgium, <sup>2</sup>Unaffiliated, Manchester, United Kingdom, <sup>3</sup>Laboratoire de Biochimie et Génétique Moléculaire, Groupe Hospitalier Cochin-Broca-Hôtel Dieu, APHP, Paris, France, <sup>4</sup>Synlab Suisse, Lausanne, Switzerland, <sup>5</sup>Laboratoire de Génétique Moléculaire, CHU Brest, Brest, France, <sup>6</sup>multiplicom NV, Niel, Belgium, <sup>7</sup>Laborarzpraxis Dres. med. Walther, Weindel und Kollegen, Humangenetik, Frankfurt, Germany, <sup>8</sup>Institut Universitaire de Recherche Clinique, Public Health - Hospital Medical School, Laboratoire de Génétique Moléculaire, Montpellier, France, <sup>9</sup>Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, <sup>10</sup>Institut für Humangenetik, Medizinische Hochschule, Hannover, Germany, <sup>11</sup>Laborarzpraxis Dres. med. Walther, Weindel und Kollegen, Humangenetik, Frankfurt, Germany, <sup>12</sup>Laboratoriumsmedizin Dortmund, Dr. Eberhard und Partner, MVZ, Molekulargenetik, Dortmund, Germany.

**Introduction:** In 1996, the Cystic Fibrosis (CF) Network organized the first large international external quality assessment (EQA) scheme for CFTR genetic testing. The scheme evolved from a paper-based six-sample EQA to automated electronic data-collection for three samples. Originally, EQA forms were distributed by fax and post, later by e-mail. Since 2004, an online submission system and database have revolutionized the workflow.

**Materials and Methods:** Participants receive purified DNA samples, since 1999 accompanied by mock clinical cases. Written reports as would be sent to the requesting clinician have to be submitted. Hence the entire analytical process is evaluated, including genotyping, report content, clinical interpretation and the correct use of HGVS nomenclature. Genotype and report results are scored against predefined criteria by a group of expert assessors. Persistent poor performers (unsuccessful in the current year and at least once in the two previous years) are notified and expert support for corrective actions is offered.

**Results:** In 1996, 35% of laboratories made genotyping errors. Within four years, this number decreased to 10%, and by 2015 it was down to 3%. The percentage of interpretation errors or risk calculation errors varies depending on the complexity of the cases. Longitudinal analysis shows that repeated participation improves clinical interpretation.

**Conclusions:** Continued EQA participation is advised to further improve testing quality. Interpretation and risk calculation are still prone to errors in more challenging cases. Consequences for poor performance are lacking and are suggested to be implemented by the government to ensure patient safety.

#### P19.09

#### Results from a survey of UK patients at risk of bowel cancer, their experiences and information preferences

S. M. A. Goodman, H. Skilton, R. Jones;  
Plymouth University, Plymouth, United Kingdom.

Individuals at increased risk of familial bowel cancer are advised to have regular colonoscopy and to discuss the implications of their diagnosis with relatives. However, only a minority of relatives access screening or genetic testing, which is partly due to lack of knowledge about their personal risk. We are studying information preferences to develop ways for patients to

#### P19.10

#### Genetic counselling in a family with Cri du Chat syndrome: beware of cryptic chromosomal rearrangements and family risks

E. Haquet, J. Puechberty, P. Blanchet, L. Pinson, M. Willems, G. Lefort, F. Pellestor, A. Schneider, S. Taviaux, D. Genevieve, C. Coubes; Hôpital Arnaud de Villeneuve, Montpellier, France.

A 31-year-old woman was seen for genetic counselling in our Genetics Centre. She had questions concerning her risk of having children with Cri du Chat Syndrome (CdCS). Her older brother had been diagnosed with Cri-du Chat syndrome, confirmed on a 46,XY,del5p karyotype done 30 years ago. CdCS is characterized by a high-pitched monochromatic cry, microcephaly, mainly severe psychomotor deficiency and other variable clinical features. The incidence is about 1:30,000 live-born infants. CdCS is usually due to de novo 5p deletion. For the brother, the diagnosis was clinically suggested by the paediatrician soon after birth and then confirmed on a standard karyotype.

Genetic counselling seemed straightforward but we nonetheless ordered chromosome studies in the patient, including FISH with a 5p subtelomeric probe. FISH analysis revealed 5p duplication / 4q deletion in our patient who has a normal phenotype.

This result led us to reconsider the information originally given to the family. We ordered parental karyotype analysis which revealed an apparently balanced t(4;5)(q35;p15.5) translocation in the mother.

CdCS patients with an inherited unbalanced chromosomal rearrangement have been reported and represent about 5% of CdCS patients. Generally speaking and especially in CdCS, when the index case's karyotype was done more than 20 years ago or when the parents have not had chromosome studies including FISH, repeat chromosome studies in the index case and parental chromosome studies are recommended.

#### P19.11

#### Deciphering variants of unknown significance in the CFTR gene

M. Poulopou, I. Fylaktou, S. Kitsiou-Tzeli, M. Tzetis;  
Department of Medical Genetics, Athens, Greece.

Cystic Fibrosis is one of the most common autosomal recessive disorders affecting Caucasians. To date, more than 2000 changes in the CFTR gene have been reported (<http://www.genet.sickkids.on.ca/>) with the clinical consequences of some still being unproven. In an attempt to provide with better genotype-phenotype correlations CFTR2 database (<http://www.cftr2.org/>) has been created. CFTR2 database provides information about what is currently known about the clinical signs and symptoms associated with specific CFTR mutations. In some cases where there is not enough clinical information the mutation is described as having varying clinical consequences. This creates confusion especially for family planning of carrier couples.

In this study we are reporting data from the Department of Medical Genetics, University of Athens, regarding the pathological consequences of the following mutations: p.Phe1052Val, c.483+3A>G, p.Met348Lys, p.Ser737Phe, p.Val920Leu, p.Arg74Trp, p.Asp1152His, p.Phe305Val and p.Tyr301Cys. These data are derived after full CFTR gene screening of 19,000 general population individuals and 800 patients.

It is evident that in the era of next generation sequencing and whole genome sequencing, unless you accumulate enough data from a big number of patients, conclusions are hard to be drawn even for a monogenic disorder.

## P19.13

### Mutation detection in custom-designed gene panel NGS is more accurate compared to whole exome sequencing (WES)

R. van den Bogaard, R. J. Florijn, M. Alders, E. J. W. Redeker, H. Bikker, R. H. Lekanne-dit-Deprez, P. Lombardi, S. van Koningsbruggen, M. M. Motazacker, F. van Ruissen, M. A. Haagmans, O. R. F. Mook, M. M. A. M. Mannens;  
Clinical Genetics, AMC, Amsterdam, Netherlands.

**Introduction:** Inherited diseases are usually associated with specific disease associated gene-sets. Targeted resequencing of such gene-sets can be applied by custom designed gene panels or by WES, the latter in combination with software selection of the genes of interest. Custom panel sequencing has the advantage that the coverage for all genes can be easily optimized.

**Materials and Methods:** We have evaluated both sequencing strategies for gene coverage, mutation calling accuracy, false positive rate and copy number variation(CNV) determination for several diseases such as lymphedema, cardiac disease, metabolic disease, dementia, eye diseases and others, each with different numbers of genes. CNV detection of 1 exon deletions were evaluated using the "hidden Markov model".

**Results:** Custom sequence captures optimized for evenly distributed coverage of genes of interest easily cover over 99% of the genes of interest with at least 30 unique reads. In comparison WES reaches a maximum of 95%. Furthermore, 1 exon deletions and duplications could be reliably identified in custom sequence captures, but not in WES. Novel identified mutations for different selected diseases will be presented.

**Conclusions:** We show that for defined set of genes, custom panel sequencing is preferred over exome sequencing because of optimal coverage, calling accuracy, lower false positive rate, less unclassified variants and secondary findings and CNV detection. In conclusion, custom panel sequencing of selected sets of genes is recommended for DNA diagnostics where highly reliable results are crucial. These custom gene panel tests can be performed at considerably lower costs.

## P19.14

### Is One Diagnosis the Whole Story? Patients with Double Diagnoses

A. Kurolap<sup>1</sup>, N. Orenstein<sup>2</sup>, I. Kedar<sup>3</sup>, M. Weisz Hubshman<sup>2</sup>, D. Tiosano<sup>4</sup>, D. Tiosano<sup>4,5</sup>, A. Mory<sup>1</sup>, Z. Levi<sup>6,7</sup>, D. Marom<sup>8,7</sup>, L. Cohen<sup>3</sup>, N. Ekhilevich<sup>1</sup>, W. Tan<sup>9</sup>, H. N. Baris<sup>1,5</sup>;

<sup>1</sup>The Genetics Institute, Rambam Health Care Campus, Haifa, Israel, <sup>2</sup>Genetics Unit, Schneider Children Medical Center, Petah Tikva, Israel, <sup>3</sup>The Raphael Recanati Genetics Institute, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel, <sup>4</sup>Pediatric Endocrinology, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel, <sup>5</sup>The Ruth and Bruce Rappaport Faculty of Medicine, Technion-Institute of Technology, Haifa, Israel, <sup>6</sup>The Early Detection and High Risk GI Cancer Service, Gastroenterology Division, Rabin Medical Center, Petah Tikva, Israel, <sup>7</sup>Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, <sup>8</sup>Pediatric A, Schneider Children Medical Center, Petah Tikva, Israel, <sup>9</sup>Division of Genetics and Genomics, Boston Children's Hospital; Harvard Medical School, Boston, MA, United States.

**Background.** Evaluating a patient in the genetics clinic aims at finding a diagnosis that would explain their full clinical presentation. In some cases the patient's diagnosis remains undefined; in others it does not explain all the patient's findings. As clinicians are often bound by a „single disorder“ paradigm, diagnosing multiple genetic conditions requires a heightened sense of awareness. We aim to raise awareness to the „double diagnosis“ phenomenon and to share our experience.

**Procedure.** Patients (n=14) were seen over the last decade at The Genetics Institutes at Rambam Health Care Campus, Haifa, Israel, Beilinson hospital and Schneider Children's Medical Center Petah Tikva, Israel, and Boston Children's Hospital, Boston, MA, USA. All patients were clinically evaluated by their geneticists, and underwent diagnostic genetic analyses (Table 1). **Conclusions.** With most patients, the primary physician is the pivotal figure that facilitates their care; he is best acquainted with the patient and should be the first to question a diagnosis that doesn't explain the patient's full clinical picture. The phenomenon of double diagnosis is bound to expand in the era of next generation sequencing, as it continues to grow in clinical, rather than just research, settings. We recommend physicians to question every diagnosis and see whether it indeed explains all of the patients' symptoms, or should they continue the evaluation for a more accurate and complete diagnosis or diagnoses.

Table 1: Genetic diagnoses of patients in our case series.				
Case	Aneuploidy	Microdeletion / Micro-duplication	Imprinting disorder	Monogenic / Molecular disorder
1	Down syndrome			Gaucher disease
2	Down syndrome			Spino muscular atrophy
3	Down syndrome			Marfan syndrome
4	Klinefelter syndrome			Neurofibromatosis

5	Turner syndrome mosaicism	Williams syndrome			
6		Williams syndrome 16p11.2 del		Lynch syndrome	
7				PTEN hamartoma syndrome	
8		Xp22.31 dup & 1p22.1 dup		Sotos syndrome	
9		16p11.2 del			Neurofibromatosis
10		16p13.11 del	Angelman syndrome		
11				Congenital adrenal hyperplasia	Suspected storage disease
12				Neurofibromatosis	Fragile X
13				Abetalipoproteinemia	Neurofibromatosis
14				Hereditary neuropathy with liability to pressure palsy	Gaucher disease

## P19.15

### Preconception carrier screening for multiple disorders: evaluation of a screening offer in a Dutch founder population

I. B. Mathijssen<sup>1</sup>, K. C. A. Holtkamp<sup>2,3</sup>, C. P. E. Ottenheim<sup>4</sup>, J. M. C. van Eeten-Nijman<sup>1</sup>, P. Lakeman<sup>1</sup>, H. Meijers-Heijboer<sup>1,2</sup>, M. C. van Maarle<sup>1</sup>, L. Henneman<sup>2,3</sup>;

<sup>1</sup>Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands,

<sup>2</sup>Department of Clinical Genetics, VU University Medical Center, Amsterdam, Netherlands, <sup>3</sup>EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

**Introduction:** Although preconception carrier screening for multiple disorders is becoming more widely available, only few studies have been done to evaluate such kind of screening. This study assessed experiences with carrier screening in a Dutch founder population in which four severe recessive disorders frequently occur.

**Methods:** In a special outpatient clinic, individuals from the founder population were offered screening for the four disorders simultaneously. Those who attended between September 2012 and June 2014 were asked to participate in our evaluation study. Questionnaire(s) were completed by 182 counselees before and after counselling, and after receiving test-results.

**Results:** Although knowledge after counselling increased ( $p<0.001$ ), still 10% (compared to 34% before counselling) mistakenly thought that there is an increased risk of having an affected child if both parents are carrier of different disorders. Most participants (97%) could recall their test-results correctly. 63% Felt worried waiting for their results, but anxiety levels returned to normal afterwards. 4% (2/52) Of the carriers felt less healthy after knowing their results. Participants did not regret testing (97%) and would recommend screening to others (97%). Eight carrier couples were identified who all made reproductive decisions based on their test-results.

**Conclusion:** Individuals from the Dutch founder population who were offered screening were very satisfied. Although familiarity with genetic diseases is high in this population, knowledge is still not optimal. No adverse psychological effects were demonstrated. Our experiences provide lessons for the implementation of expanded carrier screening panels in the general population.

**Grant:** The Netherlands Organization for Health Research and Development.

## P19.17

### How to improve parents' and children's experience of predictive testing for Familial Hypercholesterolaemia

K. Forrest Keenan, L. McKee, Z. Miedzybrodzka;

University of Aberdeen, Aberdeen, United Kingdom.

**Background:** Genetic testing is recommended in children at risk of Familial Hypercholesterolaemia (FH) to allow early treatment with statins. In Scotland a system of cascade screening was introduced in 2008. Whilst 13% of the at-risk population have been identified to date, the majority remain untested and undiagnosed, with uptake rates for children a concern. **Methods:** A qualitative study of 17 parents whose children were offered FH genetic testing and treatment.

**Results:** Key points for clinical practice were: (1) Consider if parent(s) need time to discuss how/when to share information with their child about FH, and offer separate appointment before meeting child if necessary; (2) Provide age appropriate and engaging information for parents about FH to use with children. Signpost to resources such as <http://heartuk.org.uk/FHchildrensresources>; (3) Explore any parental concerns and emotions e.g. grief, loss, guilt, fear, threat. (4) Offer referral elsewhere for additional support if necessary e.g. more experienced team members, psychology or FH nurse; (5) Explore parents and children's views about genetic testing and statin treatment - aim for joint decision making; (6) Use a multidisciplinary approach and joint clinics where possible and (7) If family is at risk of disengaging aim to maintain a good relationship and advise that your 'door is always open.' **Conclusion:** Attention to these issues in genetic counselling/paediatric genetics could improve parents and children's experiences of

predictive testing and care pathways for FH. KFK was funded by a CSO Fellowship, Scottish Government.

## P19.18

### Filling the Void: a support initiative for families affected with a rare condition living in rural/remote Australia.

**D. T. Ranaivoharison**, *D. Petrie;  
Genetic Alliance Australia, Sydney, Australia.*

**Background:** Living with a rare genetic condition and/or caring for someone with a rare genetic condition is associated with significant social and emotional impacts. These are even more pronounced for families in rural/remote areas with added difficulties of geographic isolation, limited access to services and lack of appropriate community support. Genetic Alliance Australia was created in 1988 to provide a national umbrella group for information dissemination and representation of those impacted by rare genetic conditions.

**Method:** Filling the Void (FTV) uses a collaborative approach between carers/ families and professionals to respond to the various needs of those living in isolation affected by rare genetic conditions. Individuals/ families are referred by professionals, friends, family or self-referral and contact is made via phone, email, web or social media inquiry. The outreach program is delivered through a series of rural seminars, sibling workshops, networking events and tele-counselling groups.

**Results:** FTV has been running for over 10 years. Evaluation and monitoring of the program has shown a persistent positive feedback from participants. As a response to the changing environment where information is more easily accessible, the program is looking for innovative ways to meet consumers' need. Communication with health professionals underlined the necessity to engage with early educators and allied health professionals to ensure that families' needs are met.

**Conclusion:** Rural/ remote outreach support in Australia is strongly needed. Strong collaboration is necessary to keep up with its constant evolution and its expansion to professionals.

## P19.19

### A forum for French-speaking patients with developmental diseases with no diagnosis or an ultra rare genetic diagnosis

**T. Heuyer<sup>1</sup>, C. Vicard<sup>1</sup>, A. Lapointe<sup>2</sup>, L. Demougeot<sup>2</sup>, D. Lacombe<sup>2</sup>, N. Bahi-Buisson<sup>2</sup>, S. Sigaudy<sup>2</sup>, C. Thauvin-Robinet<sup>2</sup>, L. Faivre<sup>2</sup>;**

<sup>1</sup>Maladies Rares Info Services, Paris, France, <sup>2</sup>Filière de Santé AnDDI-Rares, Dijon, France.

A diagnosis can be classically reached in 50% of patients with developmental diseases. This figure is tending to fall since the arrival of next generation sequencing, which sometimes make it possible to identify extremely rare diseases. In these two situations, patients and/or their families are very keen to share their experience with other patients confronted with the same problems. Two organizations from different horizons have joined forces to propose a solution to these patients to help them escape from their isolation. Maladies Rares Info Services (MRIS) is the reference information and support service for rare diseases in France. It offers a complete range of services (telephone, mail, ch@t, Forum...) so that families can be listened to, find support and ask questions about rare diseases or share their experience. The AnDDI-Rares network brings together reference centres, genetics laboratories and patients support groups involved in genetic developmental diseases. It is developing particular actions around the diagnosis, the management of patients, research and training/information. The patients concerned by this new offer will be informed during genetic consultations in the AnDDI-Rares network. They can visit the site of MRIS where they will find a section called forum for patients/families without diagnosis, or contact Maladies Rares Info Services if they wish to create a new forum for an extremely rare disease. A team of professionals is on hand to provide assistance and moderate. Even though international initiatives are being set up, it is important to create national initiatives to improve access for all.

## P19.20

### Developmental abnormalities and intellectual disability: an original French organisation in two national networks, AnDDI-Rares and DéfiScience

**C. Thauvin-Robinet<sup>1</sup>, L. Olivier-Faivre<sup>2</sup>, L. Demougeot<sup>1</sup>, M. Reymond<sup>3</sup>, T. Attié-Bitach<sup>4</sup>, L. Ben Slama<sup>4</sup>, L. Billette de Villemeur<sup>5</sup>, O. Boespling<sup>6</sup>, D. Bonneau<sup>6</sup>, J. Bonnefont<sup>7</sup>, L. Burglen<sup>7</sup>, P. Charles<sup>8</sup>, R. Delorme<sup>9</sup>, C. Depienne<sup>8</sup>, M. Doco-Fenzy<sup>9</sup>, M. Dupuy<sup>10</sup>, P. Edery<sup>11</sup>, C. Francannet<sup>12</sup>, D. Geneviève<sup>13</sup>, D. Germanaud<sup>13</sup>, G. Gyot<sup>6</sup>, A. Hugon<sup>5</sup>, D. Lacombe<sup>14</sup>, A. Lapointe<sup>4</sup>, S. Lyonnier<sup>7</sup>, S. Manouvrier<sup>15</sup>, I. Marey<sup>5</sup>, C. Mignot<sup>5</sup>, M. Moutard<sup>6</sup>, S. Odent<sup>16</sup>,**

*L. Pasquier<sup>16</sup>, S. Passemard<sup>4</sup>, R. Percheron<sup>3</sup>, N. Philip<sup>17</sup>, M. Poher<sup>5</sup>, M. Rio<sup>5</sup>, M. Rossi<sup>11</sup>, D. Sanlaville<sup>18</sup>, P. Sarda<sup>19</sup>, M. Tauber<sup>10</sup>, J. Thevenon<sup>1</sup>, A. Toutain<sup>20</sup>, L. Vallée<sup>21</sup>, A. Van de Velde<sup>5</sup>, A. Verloes<sup>7</sup>, C. Vernin<sup>11</sup>, DéfiScience Competence Centres, AnDDI-Rares co-coordinators, V. des Portes<sup>3</sup>, D. Héron<sup>7</sup>;*

*<sup>1</sup>AnDDI-Rares, Dijon, France, <sup>2</sup>AnDDI-Rares, DéfiScience, Dijon, France, <sup>3</sup>DéfiScience, Lyon, France, <sup>4</sup>AnDDI-Rares, Paris, France, <sup>5</sup>DéfiScience, Paris, France, <sup>6</sup>AnDDI-Rares, Angers, France, <sup>7</sup>AnDDI-Rares, DéfiScience, Paris, France, <sup>8</sup>AnDDI-Rares, DéfiScience, Strasbourg, France, <sup>9</sup>AnDDI-Rares, DéfiScience, Reims, France, <sup>10</sup>DéfiScience, Toulouse, France, <sup>11</sup>AnDDI-Rares, Lyon, France, <sup>12</sup>AnDDI-Rares, Clermont-Ferrand, France, <sup>13</sup>AnDDI-Rares, Montpellier, France, <sup>14</sup>AnDDI-Rares, Bordeaux, France, <sup>15</sup>AnDDI-Rares, Lille, France, <sup>16</sup>AnDDI-Rares, DéfiScience, Rennes, France, <sup>17</sup>AnDDI-Rares, DéfiScience, Marseille, France, <sup>18</sup>AnDDI-Rares, DéfiScience, Lyon, France, <sup>19</sup>AnDDI-Rares, DéfiScience, Montpellier, France, <sup>20</sup>AnDDI-Rares, DéfiScience, Tours, France, <sup>21</sup>DéfiScience, Lille, France.*

Developmental abnormalities (DA) and (ID) are both extremely heterogeneous groups of disorders comprising over 3,000 distinct clinical entities. Although individually rare, they affect from 1.5 to 3% of the population, corresponding to around 1.8 million French people. DA/ID, lead to chronic lifelong physical, cognitive and adaptive disability, and represents a major public health problem leading to medical and scientific challenges: diagnosis of the underlying etiology (about half of the patients remain undiagnosed), early multidisciplinary follow-up and care, substantial familial support and qualified research in various fields such as molecular biology, neurobiology, cognitive science, pharmacology, education and social science.

After the certification of reference and competence centres for rare diseases by the first "French National Plan for Rare Diseases", launched in 2005 by the French Ministry of Health, 23 national networks for rare diseases were certified in 2014 through the second plan. For DA/ID, two networks have been certified, AnDDI-Rares and DéfiScience, devoted to AD and ID, respectively. These networks gather 13 reference and 15 competence centres, all genetic diagnosis labs devoted to DA/ID, more than 40 research teams, 12 learned societies and more than 50 patients' support associations.

Taking advantage of complementary fields of expertise, AnDDI-Rares and DéfiScience work together to facilitate patients' access to functional and molecular diagnosis, implement national registries, write detailed care and follow-up recommendations, coordinate medico-social actions, teach professionals, apply communication strategies to spread knowledge and develop interactions, promote clinical and translational research, and increase international visibility and collaborations.

## P19.21

### The role of the genetic counselor in the decision by pregnant women with the risk of chromosomal abnormalities in the fetus regarding invasive diagnostics

**V. L. Izhevskaya<sup>1</sup>, L. A. Zhuchenko<sup>2</sup>;**

<sup>1</sup>Research centre for medical genetics, Moscow, Russian Federation, <sup>2</sup>Russian Medical Academy of Postgraduate Education Studies, Moscow, Russian Federation.

The effect of different demographic and social factors on the consent of pregnant women for invasive prenatal diagnosis or rejection of it was analyzed. Pregnant women from the Moscow region were examined in the period from 12.12.2012 till 30.10.2014. 1580 pregnant women were at the risk of chromosomal abnormalities in the fetus of 1: 100 and above. All of them have received genetic counseling. Invasive procedure was carried out in 1164 (73.7%) of them, 416 (26.3%) rejected it. Data on age, place of residence, social status, presence or absence of children, children's health, the presence or absence of spontaneous abortions have been received. Statistically significant differences between the two analyzed groups of women in these parameters were not obtained. The proportion of women who refused invasive procedure, significantly differed from two genetic counselors ( $\chi^2 = 7.8$ ;  $p = 0.0055$ ). First counselor's patients significantly more frequently indicated that they feared complications of invasive procedures (63.8% vs 31%), or they could not formulate reasons for the refusal of it (83.3% vs 16.7%). Second counselor's patients significantly more frequently pointed to the decision to have a child, regardless of his health (70% vs 30%). About 13% of patients of both doctors refused the procedure because they were sure that their baby is healthy. Perhaps we need additional training geneticists involved in the program of early prenatal screening. This work was supported by the Russian Foundation for Humanities, project 15-03-00822.

## P19.22

### Genetic Counselling Service in Korea

**H. J. Kim;**

*Korean Foundation for Rare Disease, Seoul, Korea, Republic of.*

Genetic Counseling Service(GCS) is considered as an integral part of medical service for pts. with genetic disease in most of developed countries. Howe-

ver, GCS is yet to be established in Korea where National Health Care System covered by the uniform national insurance policy.

Korean Governmental Health policy for pts. with Rare disease includes special discount health care fees and benefits, but genetic counseling is not recognized as an health service yet. Therefore many pt. and family with genetic disease look for help and requested aids for GCS to KFRD, an advocate for rare disease.

In May 2012 KFRD started funding for GCS to aid pts. and family members at high risk, in order to provide GCS including genetic testing and for the evaluation of effectiveness of GCS, survey questionnair was administered to 496 individuals of pts & family members participated.

Among 118 respondents to the survey, 109(92%) responded „overall satisfaction“ on GCS, 108(91.5%) „helpful in understanding the diagnosis and natural history of the disease affected“, 103(87.2%) „helpful in understanding accurate medical and genetic information“ and 98(83%) „helpful in management of disease and make decision on reproductive options“.

111(94%) acknowledged financial aids allow them to receive GCS and expressed strong desire of the aid program to be continued.

These findings strongly support that GCS should be provided to pts. and family with genetic disease for the better management and prevention of incurable and disabling rare hereditary disease, as an integral part of health care policy in Korea.

#### P19.23

#### Examining the genetics nursing lesson's situation in nursing curriculum in Turkey

*Y. Erdem, A. Güneş, N. Bora Güneş;  
Kirikkale University, Kirikkale, Turkey.*

**Background:** Genetics nurses help people at risk for or affected by diseases with a genetic component achieve and maintain health. So it is important to all university to integrate genetic nursing courses into the curriculum. In Turkey, genetic diseases are high because of consequences married.

**Aim:** To explore the nursing curriculum about genetics nursing courses in Turkey

**Methods:** In this descriptive research, 133 bachelor degree nursing schools were explored via e-mails and telephone interview.

**Results:** In this research, we found that % 2.25 (3 nursing school) of all nursing school are given separately to genetics courses into their curriculum. Two of them are included as Medical Genetic and Biology in the first year as mandatory. Also, all of them are included as Genetics Nursing courses in the 3rd years as optional. In Turkey most of bachelor degree nursing schools integrated genetics into different courses like pediatric nursing, obstetrics and gynecology nursing or medical nursing. In our country, most of nursing students taught that the courses is insufficient.

**Discussion:** With major advances in genetics and genomics, nurses need to develop their knowledge and understanding of the topic and know how to integrate this into practice.

**Conclusion:** In Turkey genetic nursing course curriculum is insufficient in the nursing bachelor's degree and should be empowered

#### P19.24

#### Predicted and reported response to genetic risk information among hypertension patients

*L. Leitsalu<sup>1,2</sup>, K. Läll<sup>1,3</sup>, Ü. Meren<sup>4,5</sup>, H. Alavere<sup>1</sup>, M. Tammaru<sup>2</sup>, K. Fischer<sup>1</sup>, P. C. Ng<sup>1,6</sup>, A. Metspalu<sup>1</sup>;*

<sup>1</sup>*Estonian Genome Center, University of Tartu, Tartu, Estonia, <sup>2</sup>Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia, <sup>3</sup>Institute of Mathematical Statistics, University of Tartu, Tartu, Estonia, <sup>4</sup>Department of Pharmacy, University of Tartu, Tartu, Estonia, <sup>5</sup>East Tallinn Central Hospital, Tallinn, Estonia, <sup>6</sup>Genome Institute of Singapore, Singapore, Singapore.*

**Introduction:** The feasibility and utility of incorporating genetic risk prediction for common chronic diseases in primary care practice are largely unknown. We examine whether genetic information elicits health behaviour changes in those at risk for chronic diseases, and compare predicted and reported effects.

**Methods:** We conducted a study in multiple Estonian medical centres. 240 men with newly diagnosed primary hypertension visited a medical centre five times over one year. In addition to the standard cardiovascular disease risk evaluation participants received genetic risk information for hypertension-related diseases. Participants filled in surveys about the expected effect of genetic risk information (predicted), about the immediate reaction to receiving genetic information, and at the end of the study to examine the long-term effects (reported).

**Results:** At the start of the study the participants had a positive attitude and

optimistic expectations towards the impact of genetic risk predictions on their medication adherence (88%) and health behaviour (92%). A majority (95%) thought sufficient amount of information was included in the report and considered the information received understandable (98%) and informative (92%). The preliminary results show that at the end of the study, 67% reported positive changes in their everyday habits.

**Conclusions:** The results remain to be analysed relative to the specific risk information received. However, the participants predicted response to genetic risk information is more optimistic than the reported response.

**Grant reference:** This research has been supported by EU Regional Development Fund through Archimedes Foundation, grant No. 3.2.1001.11-0033.

#### P19.25

#### The influence of perceived and actual risk on use of cancer genetic services in women at risk for hereditary breast cancer

*C. Ming, C. Nikolaidis, M. Katapodi;*

*Institute of Nursing Science, Faculty of Medicine, University of Basel, Basel, Switzerland.*

**Background:** Cancer genetic services (counselling and testing) are recommended for women with family history of early onset breast cancer ( $\leq 50$  years old). The Gail model can differentiate women with high susceptibility to the disease from those at average/population risk. Perceived risk has significant effects on health behaviors, yet, it is unknown whether it influences use of cancer genetic services. The study explores associations between perceived breast cancer risk and Gail risk scores with use of cancer genetic services.

**Methods:** We recruited 430 female relatives of women diagnosed with breast cancer  $\leq 45$  years old. Perceived risk was measured with one item assessing perceived chances of getting breast cancer. We calculated 5-year and lifetime Gail scores, and evaluated barriers to using genetic services.

**Results:** Participants were 43.4 ( $\pm 11.9$ ) years old; 80% self-identified as White/Other and 20% as Black. The average 5-year and lifetime Gail scores were 1.55% and 16.42%, respectively. Average perceived risk was 4.67( $\pm 1.99$ ), meaning "save as average." From 334 participants with lifetime Gail risk  $>$ average 81.1% underestimated their risk; from 74 participants with lifetime Gail risk  $\leq$ average 5.4% overestimated their risk. Only 33 women had genetic services and 13 had testing. Lifetime Gail scores were higher for those who had genetic services and (p=0.0043). Logistic regression modeling will explore predictors of using cancer genetic services.

**Conclusion:** Women with family history of early onset breast cancer have  $\geq$ average risk for the disease. Our study adds weight to understanding use of cancer genetic services and improving risk communication and management strategies.

#### P19.26

#### Gen-Equip: Equipping European Primary Care Health Professionals to Deal with Genetics

*V. Curtisova<sup>1</sup>, E. Houwink<sup>2</sup>, L. Jackson<sup>3</sup>, P. Lunt<sup>3</sup>, M. Paneque<sup>4</sup>, V. Stefansdottir<sup>5</sup>, D. Turchetti<sup>6</sup>, M. Campos<sup>7</sup>, M. Cornel<sup>8</sup>, M. Macek<sup>9</sup>, A. Kent<sup>7</sup>, H. Skilton, PL4 8AA<sup>3</sup>;*

<sup>1</sup>*Charles University, Prague, Czech Republic, <sup>2</sup>VU University Medical Centre, Amsterdam, Netherlands, <sup>3</sup>Faculty of Health and Human Sciences, Plymouth, United Kingdom, <sup>4</sup>IBMC, University of Porto, Porto, Portugal, <sup>5</sup>Landspitali Hospital, Reykjavik, Iceland, <sup>6</sup>Università di Bologna, Bologna, Italy, <sup>7</sup>Genetic Alliance UK, London, United Kingdom.*

With expansion of genetics and genomics, primary care professionals are increasingly expected to help deliver genetic services in daily patient care. This requires appropriate skills and knowledge to deliver care that addresses patient needs and maximises use of health resources. The aim of the Gen-Equip project is to improve primary care of patients with genetic/genomic conditions. Via a partnership between expert patients and specialists in adult education, primary care and genetics, we are implementing free, online education in genomic healthcare for European primary care practitioners.

To date, we have undertaken a systematic review of genetic education for primary care and developed an educational curriculum for European professionals. Expert advice indicated that an approach based on questions asked by patients in primary care was likely to be perceived as relevant to the target learner group. Ten case-based modules set in primary care have been prepared, these can be accessed online (<https://www.primarycaregenetics.org/>) by any health professional and include pre and post-test module tests. Topics include familial cancer, familial hypercholesterolemia, inherited cardiac conditions, developmental delay and reproductive issues. A series of online webinars are also available on core topics such as 'Genetic/genomic test results: what they mean for your patient'. All educational content will be available in six European languages and accredited by national professional organisations.

Assessment of educational tools relating to changes in knowledge, skills and practice is important and we will investigate short and long-term changes. Examples of learning tools and initial assessments will be given.

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## P19.27

### **Building capacity and capability in the NHS for Genomic Medicine: a national strategic approach to the underpinning educational framework**

*A. Seller<sup>1</sup>, M. Bishop<sup>1</sup>, N. Latham<sup>1</sup>, S. L. Hill<sup>2</sup>, E. V. Davison<sup>1</sup>;*

<sup>1</sup>*Health Education England, Birmingham, United Kingdom, <sup>2</sup>NHS England, London, United Kingdom.*

England's 100,000 Genomes project aims to establish a new genomic medicine service for the National Health Service (NHS) through transformation of patient and diagnostic care pathways, improving diagnosis in patients with Rare disease and Cancer, and in time realising the potential for new and effective treatments.

This ambitious project affords a unique opportunity to implement a co-ordinated approach to workforce education and development, upskilling the existing workforce and ensuring a lasting legacy of genomics embedded in mainstream clinical practise. To address this need a £20M programme of resource, capacity and capability development has been established by Health Education England's Genomics Education Programme.

To underpin the multiprofessional education and training needs of the current workforce to deliver the 100,000 Genomes Project we undertook a gap analysis, identifying nine resources to support the project pipeline. These include a range of e-learning resources including: obtaining project consent, DNA extraction and sample processing, validation and clinical reporting of the results and an online tumour assessment tool. Our MOOC on whole genome sequencing aims to reach a wider audience. Capability and capacity is being addressed through implementation and funding of new postgraduate scientist training curricula, a new NHS specialism of Clinical Bioinformatics, and a national Genomic Medicine Master's programme for NHS staff.

To date we have funded 57 postgraduate trainees, approved 240 MSc applications and >2700 learners have registered for our online courses. We will present our strategy together with data on course engagement, and evaluation of the programme's impact on workforce transformation.

## P19.28

### **Hereditary breast and ovarian cancer: Report of a case of double heterozygosity, concurrent pathogenic variants in germline BRCA1 and BRCA2**

*S. B. Margarit<sup>1</sup>, F. Benavidez<sup>1</sup>, K. Espinoza<sup>1</sup>, P. Raimilla<sup>2</sup>, F. Cádiz<sup>2</sup>, G. Repetto<sup>1</sup>;*

<sup>1</sup>*Clinica Alemana Universidad del Desarrollo, SANTIAGO, Chile, <sup>2</sup>Clinica Alemana de Santiago, SANTIAGO, Chile.*

**Introduction:** Cases of double heterozygosity caused by the presence of mutations in the BRCA1 and BRCA2 genes are unusual. To date, the vast majority of reported cases are of Ashkenazi Jewish descent being rare in other populations. Clinical presentation of double heterozygotes individuals is varied and has a similar phenotype and age of onset as carriers of a single mutation. We report a case of double heterozygosity in the Chilean population with early age of onset compared to members of the same family carriers of a single mutation.

**General Description:** Pretest genetic counseling and commercial high-risk breast cancer panel was offered in view of proband's personal history of breast and ovarian cancer and family history of cancer. Mutations in BRCA1 and BRCA2 were identified in enriched targeted genes. Post-test genetic counseling and single site mutation testing was offered to high risk family members at our laboratory in Santiago, Chile.

**Results:** Double heterozygosity was identified in the proband. In BRCA1 c.3331\_3334delCAAG (rs80357903) also known as c.3450del4 recognized as pathogenic. This mutation is a common cause of hereditary breast and ovarian cancer in Colombian ancestry. In BRCA2 c.4889C>G (rs80358711) also known as c.5117C>G is a truncating variant that creates a premature translational stop signal. Single site mutation testing was offered to 7 family members, 3 were BRCA2 carriers, 2 BRCA1 carriers and 2 family members obtained negative results for both mutations.

**Conclusion:** Detection of double heterozygosity in germline BRCA1 and BRCA2 has implications for genetic counseling and clinical management of carriers identified.

## P19.30

### **Audit of the testing strategy for inherited bowel cancer predisposition genes in the Clinical Genetics Department and Liverpool Women's Hospital**

*K. A. Eggleton;*

*Liverpool Women's Hospital, Liverpool, United Kingdom.*

Genetic testing for families who are at an increased risk of developing bowel cancer due to a genetic predisposition can be time consuming, expensive and complex. Testing can be carried out by two methods: Directly sequencing mismatch repair (MMR) genes or indirectly using immunohistochemistry (IHC) and microsatellite instability (MSI) testing on tumour tissue. At the Liverpool Women's Hospital (LWH), an individual must fit the modified Amsterdam Criteria to qualify for direct genetic testing. Alternatively, individuals that meet a less stringent criterion (Cairns *et al* guidelines) or departmental criteria may be sent for IHC/MSI testing.

Through a clinical audit of this strategy, it was established that IHC/MSI testing had a very low pick-up rate (4%) and a 6-12 month turnaround time. In addition, this testing was found to cost a minimum of £460 per sample plus £138 for methylation tests should an abnormal result be discovered. This gave a cost per positive of £7406 in 2014. In 2015, the LWH genetics department implemented a Multiplicom HNPCC-related gene panel which reduced the cost of direct gene testing to approximately £500 per sample and reduced analysis time to 6 days. Given the number of patients tested in 2014, this test would have a cost per positive of £3500.

As a result of the audit, new departmental guidelines have now been developed. Direct gene testing has now been expanded to individuals who fall within a new departmental criteria, IHC testing has been significantly restricted and MSI testing has been removed from standard practice.

## P19.31

### **„The result is a little more complicated than we expected“: Incidental findings in predictive testing**

*R. F. Pope-Couston<sup>1</sup>, K. K. Gamet<sup>1</sup>, P. Dryland<sup>2</sup>;*

<sup>1</sup>*Genetic Health Service NZ, Auckland, New Zealand, <sup>2</sup>Labplus Auckland District Health Board, Auckland, New Zealand.*

**Introduction:** Often, genetic counselling for predictive testing may not focus on the possibility of incidental or unexpected findings due to the perception that the narrow focus of genetic testing for known mutations would make those types of findings highly unlikely. In this case, due to the nature of the test platform being used, it was found that while the consultand did not carry the maternal familial BRCA1 deletion, he did carry a pathogenic duplication. This incidental finding was a novel experience for both the genetic counsellors and laboratory staff involved and required management of a number of complex issues.

**Methods:** Laboratory staff liaised with the genetics unit to develop a report which accurately reflected how the finding had been detected. Counsellors involved considered a number of ethical issues in preparation for providing the result to the consultand and working with his family. These included planning how, or if, to raise the possibility of non-paternity given the low de novo rate for BRCA mutations and the absence of a reported paternal family history of cancer.

**Outcome:** Collaborative communication between the laboratory and genetic counselling staff and between genetic counsellors and family members was the key to effectively managing this incidental finding and the flow-on effects within the family. Successful communication and support has resulted in a number of at-risk relatives coming forward for predictive testing. This case also highlighted the importance of comprehensive pre- and post-test counselling particularly as the resolution and complexity of genetic testing platforms continues to increase.

## P19.32

### **Detection of tissue mosaicism in Klinefelter and Klinefelter-like patients by using FISH and its clinical importance**

*A. M. Hassan, A. A. B. Shahin, I. Mazen, M. M. Eid, O. M. Eid, H. T. El-Bassyouni;*

*National research center, Cairo, Egypt.*

**Introduction:** Klinefelter syndrome (47,XXY) is the most chromosomal abnormality that repeatedly registered in infertile men with an incidence of 9-11% in azoospermic individuals. However there are no fixed predictive factors for testicular sperm extraction success has been established. Detection of mosaicism could be important for the clinical evaluation. In this study we have assessed the degree of mosaicism in peripheral blood and buccal mucosal cells. The fluorescence *in situ* hybridization (FISH) using centromere X,Y probe was applied on the peripheral blood and buccal cells as a sensitive technique for the detection of mosaicism.

**Materials and Methods:** 20 Patients are classified into 12 Klinefelter syndrome patients (group I) and 8 Klinefelter syndrome-like patients (group II) according to karyotype and clinical appearance. Karyotype for each patient was done and FISH analysis for cells from peripheral blood and buccal swap was recorded.

**Results:** Out of the 12 klinefelter syndrome patients (group I), 5(41.6%) were found to have mosaic cell lines. The mosaic Klinefelter syndrome patients were 47,XXY/46,XY. Testicular volumes in patients of group II were larger when compared to those in group I. One patient in group II has no azoospermia.

**Conclusions:** The mosaicism level in testicular tissue, not only concerning germ cells but also for somatic cells, could be of relevance to the final outcome of spermatogenesis in patients with Klinefelter syndrome. So the complete evaluation should include cells from the peripheral blood and another tissue. Mucosal cells can be of help for better estimate of the sertoli cell mosaicism.

### P19.33

#### Management of emergency care for patients with FBN1 gene mutation: epidemiological study

**L. Boidron, A. Charpin, R. Tfifha, E. Gautier, C. Binquet, H. Devilliers, A. Soudry, A. Mosca-Boidron, N. Jean, B. Mangola, J. Eicher, L. Faivre;**  
Dijon University Hospital, Dijon, France.

**Introduction:** Marfan syndrome (MFS) is characterized by a variable combination of cardiovascular, skeletal or eye events. In emergencies situations, the knowledge of this history is crucial to ensure quality of care. When calling to Emergency Medical Call Center (EMCC), the patient has an interview with a medical dispatcher that adjusts the response given the gravity of the situation and the patient's history.

**Materials and Methods:** In this study we sought to evaluate the frequency, cause, and the quality of care for patients with FBN1 gene mutation calling to EMCC. Between April 2008 and October 2015, we collected all calls of the 37 mutated patients known. We analyzed the number, recurrence and reasons of call as well as the knowledge of the history of MFS by the dispatcher. **Results:** Among the 37 patients known as MFS carrier, 16 have called 25 times to EMCC during 7 years. More than half of calls (60%) had a potential link with the MFS (10 calls for chest pain, 5 for dyspnea 5). Trauma represents 5 calls. The notion of MFS was known in two calls (8%). Dispatcher's decision was appropriate in 40% of cases.

**Conclusion:** Half of patients with FBN1 mutation have used the EMCC mostly for reasons related to their condition. The notion of MFS was known only in 8% of calls that probably reduce quality and safety of healthcare. A registration of MFS patients on a special list for the EMCC should improve the quality of management.

### P19.35

#### MedGen: Development of medical genetics education through curriculum reforms and establishment of training programs

**D. Babikyan<sup>1,2</sup>, H. Skirtoun<sup>3</sup>, Z. Sedlacek<sup>4</sup>, M. Seri<sup>5</sup>, K. Sargsyan<sup>6</sup>, A. Hovnanian<sup>7</sup>, S. Allon Shalev<sup>8</sup>, E. Shalev<sup>9</sup>, L. Basel<sup>9</sup>, M. Berkenstet<sup>9</sup>, K. B. Avraham<sup>9</sup>, K. Hovhannesian<sup>10</sup>, G. Shahsuvaryan<sup>10</sup>, S. Midyan<sup>1</sup>, H. Hayrapetyan<sup>1</sup>, A. Hovhannisan<sup>1</sup>, T. Sarkisian<sup>1,2</sup>,**  
<sup>1</sup>Yerevan State Medical University after Mkhitar Heratsi, Yerevan, Armenia, <sup>2</sup>Center of Medical Genetics and Primary Health Care, Yerevan, Armenia, <sup>3</sup>University of Plymouth, Plymouth, United Kingdom, <sup>4</sup>Charles University in Prague, Prague, Czech Republic, <sup>5</sup>Alma Mater Studiorum Università di Bologna, Bologna, Italy, <sup>6</sup>Medical University of Graz, Graz, Austria, <sup>7</sup>Université Paris Descartes, Paris, France, <sup>8</sup>Technion-Israel Institute of Technology, Haifa, Israel, <sup>9</sup>Tel Aviv University, Tel Aviv, Israel, <sup>10</sup>Russian-Armenian University, Yerevan, Armenia.

Despite increasing awareness of public health officials and medical professionals about the role of genetic factors in health and diseases in Armenia and Israel, the development and implementation of effective medical genetic interventions is critically constrained by a lack of professional workforce capacity. For this reason, MedGen project has been launched where the wider objective is the development and implementation of education programs of Medical Genetics (MG) corresponding to EU recommendations of educational and professional standards in MG and reinforcement of international cooperation capacity. Four different education programs are being developed in four universities of Armenia and Israel in Genetic Counselling and Clinical Genetics. National consultations with presentation of policy briefs for MG to stakeholders provided better opportunity to develop graduate profiles and content of education programs with blended teaching and learning platform. The faculty training provided by five outstanding European universities upgrade the teaching capacity of Armenian and Israeli teaching staff to deliver the MG courses corresponding to EU standards. Accreditation and delivery of developing programs are expected by the end of 2016

with the development of cooperation between the partners and European universities as strong indicators of the project's sustainability. The harmonized education in MG will serve as a hub for international education and research programs and will contribute to the overall quality of healthcare in Armenia and Israel.

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Technion-Israel Institute of Technology	Israel
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Plymouth University	United Kingdom
Charles University in Prague	Czech Republic
Medical University of Graz	Austria
Université Paris Descartes	France
European Centre for Knowledge and Technology Transfer	Belgium

### P19.36

#### Characterization of individuals at high risk of developing melanoma in Latin America: bases for genetic counseling in melanoma

**C. Badena<sup>1,2</sup>, M. Potrony<sup>1</sup>, F. Cuellar<sup>3,4</sup>, J. Puig-Butille<sup>1,2</sup>, C. Carrera<sup>2,2</sup>, P. Aguilera<sup>3</sup>, J. Malvehy<sup>3,2</sup>, E. Nagore<sup>5,6</sup>, Z. Garcia-Casado<sup>7</sup>, C. Requena<sup>5</sup>, R. Kumar<sup>9</sup>, G. Landman<sup>9,10</sup>, B. Costa Soares de Sa<sup>10</sup>, G. Gargantini Rezze<sup>10</sup>, L. Facure<sup>10</sup>, A. Ribeiro de Avila<sup>10</sup>, M. Achatz<sup>10</sup>, D. Carraro<sup>10</sup>, J. Pedreira Duprat Neto<sup>10</sup>, T. C. Grazziotin<sup>11</sup>, R. R. Bonamigo<sup>11</sup>, M. W. Rey<sup>11</sup>, C. Balestrini<sup>12</sup>, E. Morales<sup>13</sup>, M. Molgo<sup>14</sup>, R. Marchiori Bakos<sup>15</sup>, P. Ashton-Proalla<sup>15</sup>, R. Giugliani<sup>15</sup>, A. Larre Borges<sup>16</sup>, V. Barquet<sup>16</sup>, J. Perez<sup>16</sup>, M. Martinez<sup>16</sup>, H. Horacio<sup>17,18</sup>, E. Cohen Saban<sup>17,19</sup>, C. Latorre<sup>18</sup>, B. Carlos-Ortega<sup>19</sup>, J. C. Salas-Alanis<sup>20</sup>, R. Gonzalez<sup>21,22</sup>, Z. Olazaran<sup>22</sup>, S. Puig<sup>3,23,24</sup>,**

<sup>1</sup>Melanoma Unit, Biochemistry and Molecular Genetics Department, Hospital Clínic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Barcelona, Spain,

<sup>2</sup>Centro Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Barcelona, Spain, <sup>3</sup>Melanoma Unit, Dermatology Department, Hospital Clínic & IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer),, Barcelona, Spain, <sup>4</sup>Consejo Nacional de Ciencia y Tecnología (CONACYT), Ciudad de México, Mexico, <sup>5</sup>Department of Dermatology, Instituto Valenciano de Oncología, Valencia, Spain, <sup>6</sup>Universidad Católica de Valencia, Valencia, Spain, <sup>7</sup>Molecular Biology Unit, Instituto Valenciano de Oncología, Valencia, Spain, <sup>8</sup>Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany, <sup>9</sup>Escola Paulista de Medicina - UNIFESP, São Paulo, Brazil, <sup>10</sup>International Research Center, AC Camargo Cancer Center, São Paulo, Brazil, <sup>11</sup>Dermatology Department and Post-Graduation Program of Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Brazil, <sup>12</sup>Servicio de Dermatología, Hospital Dr. Sótero del Río, Santiago de Chile, Chile, <sup>13</sup>Servicio de Dermatología, Hospital San Juan de Dios, Santiago de Chile, Chile, <sup>14</sup>Pontificia Universidad Católica de Chile, Santiago de Chile, Chile, <sup>15</sup>Department of Dermatology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, <sup>16</sup>Unidad de Lesiones Pigmentadas, Cátedra de Dermatología, Hospital de Clínicas

**Introduction:** CDKN2A is the main high-risk melanoma-susceptibility gene, but has been poorly assessed in Latin America. We analyzed CDKN2A and MC1R in patients from Latin America with familial and sporadic multiple primary melanoma (SMP) and compare the data with those for patients from Spain to establish bases for melanoma genetic counseling in Latin America.

**Material and Methods:** CDKN2A and MC1R were sequenced in 186 patients from Argentina, Brazil, Chile, Mexico, and Uruguay, and in 904 Spanish patients. Clinical and phenotypic data were obtained.

**Results:** Overall, 24 and 14% of melanoma-prone families in Latin America and Spain, respectively, had mutations in CDKN2A. Latin American families had CDKN2A mutations more frequently ( $P = 0.014$ ) than Spanish ones. Of patients with SMP, 10% of those from Latin America and 8.5% of those from Spain had mutations in CDKN2A. Latin American patients had fairer hair and skin and a higher prevalence of MC1R variants compared with Spanish patients.

**Conclusion:** The inclusion criteria for genetic counseling of melanoma in Latin America may be the same criteria used in Spain, as suggested in areas with low to medium incidence, SMP with at least two melanomas, or families with at least two cases among first- or second-degree relatives.

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**P19.37****Molecular genetics for the patients - prioritization criteria in the face of limited resources**M. Puiu<sup>1,2</sup>, N. Andreescu<sup>1</sup>, S. Farcas<sup>1</sup>, C. Zimbru<sup>2</sup>, A. Chirita-Emandi<sup>1</sup>;<sup>1</sup>University of Medicine & Pharmacy Victor Babes, Genetics Department, Center of Genomic Medicine, Timisoara, Romania, <sup>2</sup>Regional Center of Medical Genetics Timis, Emergency Hospital for Children "Louis Turcanu", Timisoara, Romania, <sup>2</sup>Department of Automation and Applied Informatics, Faculty of Automation and Computers, Politehnica University, Timisoara, Romania.

**Background:** The technology for genomic studies has expanded tremendously in the last decade. Although the price of molecular genetic tests has gradually decreased, the burden of costs is considerable even for highly developed European countries. Romania is a developing country where even though appropriate laboratory equipment now exists in some genetic centres, the cost for consumables needs to be well planned. We aimed to define an approach for prioritizing the allocation of molecular genetics tests at national level.

**Methods:** The norms for allocating resources need to account for clinical usefulness of a test(treatment/prevention strategies); and treating people equally. With limited resources, the degree of an individual's need for medical intervention may be the most important criterion. Nonetheless, it is imperative to offer genetic tests non-discriminatively to all people in need, regardless of their economical/social/ethnic status.

**Results:** Romanian Society of Medical Genetics together with the Ministry of Health, Romanian National Alliance for Rare Diseases(RoNARD) and other stakeholders are developing a national strategy for allocating resources, based on epidemiological data on genetic conditions in Romania. In accordance with needs, healthcare expertise and existing infrastructure, prioritization criteria are being developed to optimize patient access to genetic tests. Coordination between regional centres of medical genetics is crucial for improved efficiency.

**Conclusion:** Further work is needed to determine the criteria necessary in decision making, in order to respect equality but also clinical need within a limited budget.

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**P19.38****Using MOOCs to increase exposure to genomics in an undergraduate medical curriculum**

L. Marks, M. Jackson, S. Meek;

University of Glasgow, Glasgow, United Kingdom.

**Introduction:** We have recently found that 'Student Selected Component' (SSC) provision in our Medical School undergraduate curriculum needed to be expanded to exemplify the diversity of modern medicine, particularly in the area of genomics. Simultaneously we have also run a MOOC 'Cancer in the 21st Century: The Genomic Revolution' and as educators and course designers we were frustrated by the seeming redundancy facing an excellent example of course design and implementation.

**Methods:** Earlier this year we re-purposed the MOOC, using a blended learning approach, in order to offer a Cancer Genomics SSC to a wider group of students than previously possible. To evaluate its success we used a combination of a questionnaires and thematic analysis of student reflective diaries as well as staff evaluations.

**Results:** Analysis of the questionnaires and reflective diaries highlighted a number of key themes. The overall level of enjoyment of the SSC was high but a number of issues deserve further investigation. In particular we will discuss knowledge versus skill acquisition, pacing of the content, interaction with fellow students and educators and the concept of learning along side 'non-experts'.

**Discussion:** Genomics is a key area for the next generation of medical students to have a secure understanding of, but the ever crammed undergraduate curriculum as well as the reliance on 'in house' expertise may limit the exposure to this exponentially expanding area. We propose that the use of MOOCs may be a novel and effective way to combat this challenge.

**P19.39****Innovative Strategy for Achieving Optimal Care and Significant Cost Savings for Critically Ill Neonates**

V. Ortega, G. V. N. Velagaleti;

University of Texas Health Science Center, San Antonio, TX, United States.

Among the yearly 4 million newborns in the US, 15% are premature accounting for 75% of NICU admissions while the remaining 25% term babies ad-

mitted have varying pathology including developmental delay/intellectual disability (DD/ID). Establishing underlying genetic etiology early helps with diagnosis, prognosis and comorbidity information. According to American Academy of Pediatrics chromosome analysis (CA) is required for evaluation of children with DD/ID. Yet CA and FISH are sub-optimal in identifying the etiology of DD/ID. American Academy of Neurology, Child Neurology Society, and American College of Medical Genetics recommend that chromosomal microarray analysis (CMA) be the first-line genetic test. However, the average turnaround time for CMA is 21 days and the daily NICU cost exceeds \$3,500 per infant. With test utilization and costs significantly impacting healthcare institutions, we developed a novel approach to perform CA with reflex testing to CMA on pediatric blood samples. A CA preliminary result is reported within 24 hours. If positive for trisomy, final result is reported within 48-72 hours. If negative, CMA is reflexed based on practice guidelines. Clients are only charged either CA or CMA. Since inception in January 2015, clients requested reflex testing on 43 patients, of which 6 resulted in a positive prelim requiring no further testing. With our approach, we estimate a cost savings of ~\$63,000 per infant by reducing the NICU stay by ~16-18 days. Reflex testing to CMA after a 24 hour CA preliminary study shows potential for extensive cost savings while maintaining optimal care for NICU patients.

**P19.40****The Italian "Telethon Undiagnosed Diseases Program"**

R. Castello<sup>1</sup>, A. Torella<sup>2,1</sup>, F. Musacchia<sup>1</sup>, M. Mutarelli<sup>1</sup>, M. Dionisi<sup>1</sup>, G. Oliva<sup>1,3</sup>, M. Pizzo<sup>1</sup>, F. Radio<sup>4</sup>, M. Dentici<sup>4</sup>, A. Bartuli<sup>4</sup>, M. Digilio<sup>4</sup>, A. Ciolfi<sup>4</sup>, S. Pizzi<sup>4</sup>, A. Bruselles<sup>5</sup>, V. Caputo<sup>6</sup>, G. Mancano<sup>7</sup>, S. Maitz<sup>7</sup>, G. Sgroi<sup>7</sup>, G. Cappuccio<sup>8,1</sup>, M. Pinelli<sup>9</sup>, E. Rizza<sup>9</sup>, G. Parenti<sup>9,1</sup>, S. Banfi<sup>2,1</sup>, M. Tartaglia<sup>4</sup>, A. Selicorni<sup>7</sup>, N. Brunetti-Pierri<sup>8,1</sup>, B. Dallapiccola<sup>4</sup>, L. Monaco<sup>9</sup>, V. Nigro<sup>2,1</sup>, <sup>1</sup>Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy, <sup>2</sup>Seconda Università di Napoli, Napoli, Italy, <sup>3</sup>ICAR-CNR, Napoli, Italy, <sup>4</sup>Ospedale Pediatrico Bambino Gesù - IRCCS, Roma, Italy, <sup>5</sup>Istituto Superiore di Sanità, Roma, Italy, <sup>6</sup>Università di Roma „La Sapienza”, Roma, Italy, <sup>7</sup>MBBM Foundation- AO San Gerardo, Monza, Italy, <sup>8</sup>Università di Napoli Federico II, Napoli, Italy, <sup>9</sup>Fondazione Telethon, Milano, Italy.

Undiagnosed genetic disorders represent a significant burden for affected children and their families. The lack of diagnosis precludes the development of specific therapies and genetic counselling. The mission of Italian Telethon requires aggregating all available resources for the benefit of genetic patients. To this purpose, the Telethon Undiagnosed Diseases Program has been launched in February 2016.

The Program has two major aims: 1) to establish a shared and standardized clinical selection of undiagnosed children through comprehensive phenotyping; 2) to identify genetic causes of undiagnosed genetic diseases by using NGS.

The Program started in February 2016 as a three-year pilot plan. From April 2016, Italian physicians will be able to submit potential cases of undiagnosed disorders through a specifically developed web tool. The program is centered at the Telethon Institute of Genetics and Medicine (Pozzuoli) where NGS activities will be converged. It will rely on a core network of three clinical centers: Ospedale Pediatrico Bambino Gesù (Rome), Ospedale San Gerardo-Fondazione MBBM (Monza) and AOU Federico II (Naples). These centers will be working together for phenotyping and selecting the cases. Family members will be analyzed by high-coverage whole exome sequencing. Results will be shared and compared with those from similar international sequencing projects to identify additional patients with the disease/molecular defect. Within a time-frame of three years, we will sequence 350-400 families (1200-1500 individuals). A particular effort will be made in order to standardize all patients' related information by adopting internationally standard tools such as Phenotips and Phenome Central.

**P19.41****Genetic counselling for NIPT - a South African perspective**M. Schoeman<sup>1</sup>, M. F. Urban<sup>1</sup>, S. Morris<sup>2</sup>;<sup>1</sup>Stellenbosch University / Tygerberg Hospital, Cape Town, South Africa, <sup>2</sup>Fetal Assessment Centre, Cape Town, South Africa.

**Introduction:** Non-invasive Prenatal Testing (NIPT) using cell-free fetal DNA was offered in South Africa for the first time by our unit in July 2013. We describe the first 2 years of our experience with implementing NIPT in a clinical, private practice setting, specifically focusing on the genetic counselling considerations. **Materials and Methods:** A record review was performed for data pertaining to women who received genetic counselling for NIPT during the period July 2013 - June 2015 for common autosomal and/or sex chromosome aneuploidies and/or gender. Data was collected on rate of uptake of NIPT, indications and motivations for testing, characteristics of patients and test results. **Results:** During this period, 385 women received genetic counselling about NIPT and 354 (91%) accepted the test. Of wo-

men tested, 79% were at high risk for Down syndrome, because of advanced maternal age (60%) or positive screening tests (21%). Mean maternal age was 38 years and tests were mostly performed between 12-13 weeks gestation. Eight (2.3%) NIPT screen positive results were obtained: seven for Trisomy 21 and one for Trisomy 18, seven ended in termination of pregnancy. Conclusions: In South Africa, NIPT is a relatively expensive screening test, currently only available in the private sector to a select group who can afford it. Whilst most women requested NIPT for a high risk of Down syndrome, other reasons included additional reassurance or general concerns about Down syndrome/disability. Pre-test counselling is important- NIPT is declined in 9% of cases, often because the indications for NIPT are misunderstood.

#### P19.42

#### Implementing non-invasive prenatal testing for aneuploidy in a national healthcare system: global challenges and national solutions

R. V. Van Schendel<sup>1,2</sup>, C. G. Van El<sup>1,2</sup>, E. Pajkrt<sup>3</sup>, L. Henneman<sup>1,2</sup>, M. C. Cornel<sup>1,2</sup>,

<sup>1</sup>VU University Medical Center, Amsterdam, Netherlands, <sup>2</sup>EMGO Institute for Health and Care Research, Amsterdam, Netherlands, <sup>3</sup>Academic Medical Centre, Amsterdam, Netherlands.

**Background:** Since the introduction of non-invasive prenatal testing (NIPT) in 2011, mainly by commercial companies, a growing demand for NIPT from the public and healthcare professionals has put pressure on healthcare systems of various countries. This study identifies the challenges of establishing a responsible implementation of NIPT for aneuploidy in prenatal healthcare, by looking at the Netherlands.

**Materials and methods:** We used a mixed methods approach involving 13 stakeholder interviews (laboratory specialists, gynecologists, clinical geneticists, midwives, patient organization representatives, health-insurance advisor and prenatal screening experts), document analysis and (participatory) observations of the Dutch NIPT consortium meetings. The Diffusion of Innovation Theory and a Network of Actors model were used to interpret the findings.

**Results:** Implementation of NIPT was facilitated by several factors. The set-up of a national NIPT Consortium enabled discussion and collaboration between stakeholders. Moreover, it led to the plan to offer NIPT through a nationwide research setting (TRIDENT study; Trial by Dutch laboratories for Evaluation of Non-Invasive Prenatal Testing), which created a learning phase for careful implementation. The Dutch legal context was perceived as a delaying factor, but eventually gave room for the stakeholders involved to organise themselves and their practices.

**Conclusions:** This study shows that implementing advanced technologies with profound effects on prenatal care benefit from a learning phase that allows time to carefully evaluate the technical performance and women's experiences and to enable public debate. Such a coordinated learning phase, involving all stakeholders, will stimulate the process of responsible and sustainable implementation.

#### P19.43

#### A systematic review of interventions to provide genetics education for primary care

M. Panegue<sup>1</sup>, D. Turchetti<sup>2</sup>, L. Jackson<sup>3</sup>, P. Lunt<sup>4</sup>, E. Houwink<sup>5</sup>, H. Skirton<sup>3</sup>,

<sup>1</sup>IS3, Instituto de Inovação e Investigação em Saúde, Porto, Portugal, <sup>2</sup>Department of Medical and Surgical Sciences, Unit of Medical Genetics, University of Bologna, Bologna, Italy, <sup>3</sup>Faculty of Health and Human Sciences, Plymouth University, PL4 8AA, Plymouth, United Kingdom, <sup>4</sup>Faculty of Health and Human Sciences, Plymouth University, PL4 8AA, Plymouth, United Kingdom, <sup>5</sup>Department of Clinical Genetics, Section Community Genetics, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

At least 10% of patients seen in primary care have a condition with a genetic component. Previous studies indicate that primary care practitioners lack practical knowledge of genetics and genetic testing and lack confidence in providing services related to genetic conditions. There is therefore strong potential for professionals to fail to recognise patients who are at risk of genetic disease, resulting in inadequate management or referral to specialist services. The aim of this systematic review was to evaluate genetics educational interventions in the context of primary care.

We used the process for systematic reviews developed by the Centre for Reviews and Dissemination and conducted a search of five relevant electronic databases. Primary research papers were eligible for inclusion if they included data on outcomes of educational interventions for primary care professionals focussed on genetics. The results from each paper were coded and presented in narrative form.

Eleven studies were included in the review. The five major themes identified were: prior experience, changes in confidence, changes in knowledge, chan-

ges in practice, satisfaction and feedback. In five studies, knowledge of practitioners improved following the educational programmes, while practitioner confidence improved in six studies. There was little apparent change to practice, where this was investigated.

The objective of educational programmes is to enhance patient care, but more effort is needed to measure resulting changes in practice. We also suggest that, in addition to educational programmes, provision of resources to supply 'just in time' information and accessible clinical tools may contribute to improving care.

#### P19.44

#### Challenging the Cancer Genetic Counselling Units (CGCU) with the Treatment Focused Genetic Testing (TFGT) for Ovarian Cancer (OC) patients: the ICO network experience

À. Teulé<sup>1</sup>, A. López<sup>2</sup>, L. Feliusbadó<sup>3</sup>, H. Ahmed<sup>3</sup>, E. Darder<sup>2</sup>, A. Izquierdo<sup>2</sup>, A. Solanes<sup>4</sup>, E. Tornero<sup>4</sup>, M. Navarro<sup>4</sup>, M. Menendez<sup>4</sup>, E. Grau<sup>4</sup>, S. Iglesias<sup>4</sup>, M. Salinas<sup>4</sup>, A. Velasco<sup>2</sup>, G. Capellà<sup>1</sup>, C. Lázaro<sup>1</sup>, P. Barretina<sup>2</sup>, J. Brunet<sup>2,4,5</sup>,

<sup>1</sup>Heredity Cancer Program, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain, <sup>2</sup>Heredity Cancer Program, Catalan Institute of Oncology, Girona, Spain,

<sup>3</sup>Medical Oncology Department, Catalan Institute of Oncology, Girona, Spain, <sup>4</sup>Heredity Cancer Program, Catalan Institute of Oncology, Badalona, Spain.

Since the approval of the PARP inhibitor olaparib, the TFGT indications for OC have increased. Furthermore, several guidelines include genetic testing for all non-mucinous OC patients irrespectively of age and family history. All these new indications challenged our CGCU and Molecular Diagnosis Laboratory (MDL).

In December 2014, the Gyne Oncologists Team (GOT), CGCU members and the MDL established a consensus protocol on indications for risk assessment (RA) and TFGT. An express track for RA at CGCU/CFU (0-10 days) and test results delivery (<35 days) was defined for patients whose germline BRCA1/2 test results were needed to tailor their treatment.

Between January 1st and November 30th, 115 new OC patients were seen at the CGCU/CFU. 27 (23%) followed the express track. Median time from the referral date to test results for standard GC was 77 days (36-98) and 27 days (18-65) for express GC. Median age was 59 (23-80) and 63% had not family history of cancer. BRCA1/2 results were available for 100 patients. deleterious mutations and USV were identified in 22% (15% in the group without family history of cancer) and 9% of patients respectively.

The development of an express GC track for OC patients with a potential olaparib indication allowed us to offer RA and TFGT through our CGCU thus avoiding direct testing from the GOT. Time to first appointment for RA and test results was optimal. The relatively high frequency of BRCA1/2 mutations could be explained by the bias selection of patients with platinum sensitive relapse.

#### P19.45

#### Clinical features associated with pericentric inversion of chromosome 9 [inv(9)(p12q13)]

G. C. Cozaru, M. Aschie, A. Mitroi, D. Capatina, C. Brinza;

„Sf. Apostol Andrei” Constanta County Emergency Clinical Hospital, CONSTANTA, Romania.

**Introduction:** This paper presents a detailed study of inversion in chromosome 9 and its correlation with phenotype features. The inv(9)(p12q13) has been reported in various human diseases such as couples with repeated spontaneous abortions, bad obstetric history, infertility and congenital anomalies.

**Aim:** In this study we aimed to evaluate the clinical impact of inversion by presenting three case studies: 2 children diagnosed with dysmorphic features and congenital anomalies and one couple with infertility. So, first case (5 days/F) has: facial dysmorphism, macrocephaly, cleft lip palate, low set ears, limb shortening, right pulmonary hypoplasia; second case (2 days/F) present: short stature, dysmorphic features, hypertelorism, low set ears, short neck, mild hypotonia; and the third case is a couple (F = 37 yrs / M = 39 yrs) with two pregnancies stopped (in the first trimester) and without a family history of reproductive disorders.

**Materials and Methods:** In this respect, we made karyotyping analysis using GTG-banding for each proband and also for their parents.

**Results:** Parental origin of inv(9)(p12q13) was detected in maternal (in the second case) in one children and in the other two cases was *de novo* origin (first case and the female).

**Conclusion:** Our investigation revealed that all three cases with inv(9)(p12q13) had various clinical features and we appreciate that inv(9) may have a role in the abnormal phenotype development. The parental chromosomal analysis is essential for appropriate genetic counseling.

**P19.46****Nurses and midwives attitude toward pre-conception carrier screening (PCS) in Japan**

A. Kondo<sup>1</sup>, D. Nakao<sup>1</sup>, M. Murakami<sup>1</sup>, K. Mori<sup>1</sup>, M. Morine<sup>1</sup>, K. Hinokio<sup>1</sup>, T. Iwai<sup>1</sup>, K. Takahashi<sup>2</sup>, S. Izumi<sup>2</sup>, K. Maeda<sup>1</sup>;

<sup>1</sup>Shikoku Medical Center for Children and Adults, NHO, Zentsuji, Japan, <sup>2</sup>Tokai University School of Medicine, Isehara, Japan.

**Introduction:** It is known that identifying carriers of recessive disorders before pregnancy has the potential to benefit couples. In Japan, PCS has not been introduced yet. However, a number of commercial companies are offering PCS directly-to-consumers, recently. In this study, we would like to find out the view of the health care providers on PCS in Japan

**Materials and Methods:** A total of 24 nurses under an adult female ward (AF) and 38 nurses/midwives under a prenatal maternity ward (PM) at a tertiary hospital responded to the questionnaire based on both the Likert scale method and free writing. Average age of nurses/midwives were 32.9(AF) / 32.9(PM) year old respectively and all of them were female.

**Results:** 65% of the participants answered genetics is difficult and 16% answered genetics is scary. 68% of the participant had a wish to have PCS for themselves. There were no significant difference between the ward (AF/PM) and their specialty. However, unmarried group tended to wish having PCS for themselves and their partners compared to married group ( $p<0.005$ ). As their free comment, they showed the manner to respect a person's willingness. Some people mentioned a concern about discriminations for the carrier.

**Conclusion:** Surprisingly, Japanese nurses and midwives are positive for PCS. It could be related to recent science reports and media regarding genetic therapy or possible preventions. Some participants pointed out concerns and issues around PCS in the future. It would be important to discuss on these issues before future introduction of PCS in Japan.

**P19.47****Public involvement in policy making for newborn screening: goals, definitions, mechanisms, levels, and evaluation**

M. E. Jansen<sup>1,2</sup>, C. G. van El<sup>2</sup>, M. C. Cornel<sup>2</sup>;

<sup>1</sup>Institute for Public Health Genomics, School for Oncology and Developmental Biology (GROW), Faculty of Health, Medicine, and Life Sciences, Maastricht University, Maastricht, Netherlands, <sup>2</sup>Department of Clinical Genetics, Section Community Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands.

Technological developments have influenced newborn bloodspot screening (NBS) in the past and will probably influence the purpose of programs now and in the future. A shift in the purpose of NBS has implications for the public. However, the public is not structurally involved in NBS policy decisions. In light of current discussions about expanding NBS via Next Generation Sequencing (NGS), an approach to involve the public is needed for meaningful and successful public health policy development.

To summarize different key elements for public involvement in policy making for NBS, a rapid review of literature was performed. Search terms included: newborn, screening, public involvement, genetics, and decision making. Furthermore, a snowball method was applied where the references of resultant key articles were checked for additional relevant articles.

Five key topics were summarized from literature: the goals of public involvement, how to define 'public', which mechanisms exist for public involvement, on what levels it can prove to be valuable, and relevant indicators to evaluate the outcome. Most literature focused on public involvement in general, and the literature discussing NBS illustrated that the involvement is often through patient representatives, takes place *ad hoc*, and focuses on informing rather than deliberating.

Public involvement does not take place in a structured or transparent manner in current NBS policy making. It is relevant to shape such involvement and develop a model to include relevant views from the public, and also debate whether expanding NBS is the right mechanism to implement possibilities from NGS.

**P19.48****Families in which the rare disease is not rare - 3 case reports**

R. Gaillyová<sup>1,2</sup>, I. Valášková<sup>1,2</sup>, J. Němečková<sup>1</sup>, R. Beharca<sup>1</sup>, H. Bučková<sup>3</sup>, A. Holčíková<sup>4</sup>, J. Blatný<sup>5</sup>, L. Foretová<sup>6</sup>;

<sup>1</sup>University Hospital, Dept. of Medical Genetics, Brno, Czech Republic, <sup>2</sup>Faculty of Medicine, Masaryk University, Brno, Czech Republic, <sup>3</sup>University Hospital, Pediatric Clinic, Brno, Czech Republic, <sup>4</sup>University Hospital, Clinic of Pediatric Infectious Diseases, Brno, Czech Republic, <sup>5</sup>University Hospital, Department of Children Hematology, Brno, Czech Republic, <sup>6</sup>Masaryk Memorial Cancer Institute, Dept. of epidemiology and tumor genetics, Brno, Czech Republic.

The Department of medical Genetics of University Hospital in Brno (Czech Republic) is closely involved in the National Strategy for rare diseases in the country. We actively cooperate with the Coordinating center for rare diseases in the Czech Republic at the University Hospital Prague Motol and we are also members of teams of medical professionals for patients with Epidermolysis bullosa congenita (EB Centre) and for patients with Cystic fibrosis (CF Centre) at the University Hospital in Brno. We introduce three cases of families, where the patient combine several rare diseases. As a part of the CF center, we have a patient in our care, who is treated simultaneously for Cystic fibrosis and Hemophilia A. Together with the EB Center at the University Hospital Brno, we take care about the family with recurring Epidermolysis bullosa and Marfan syndrome. Our patient, who suffers from Huntington's Chorea is also a carrier of the BRCA gene mutations. She underwent cancer treatment and is also a carrier of Duchenne muscular dystrophy (DMD). Her two sons died prematurely as a result of DMD. Molecular genetic testing, genetic counseling and genetic prevention in these families are an important part of health care.

**P19.49****A new model to represent medical laboratories in Orphanet adapted to genomic developments in a changing international context**

M. Arles, V. Lanneau, B. Belloir, Q. Laurent, S. Gérard, M. Hanauer, A. Rath; Inserm, US14 - Orphanet, Paris, France.

For patients affected by a rare disease, obtaining a timely and accurate diagnosis is key to accessing the appropriate medical expertise. Orphanet, the reference database for rare diseases and orphan drugs, offers a range of freely accessible services, including a directory of medical laboratories and diagnostic tests. In order to respond to the evolution of genetic testing techniques and to provide useful information for the identification of laboratories across Europe in the framework of the European Cross-Border Healthcare Directive, Orphanet has further developed its medical laboratories and clinical tests database. New information has been associated with the tests, with each test assigned at least one purpose (e.g. prenatal, postnatal, preimplantation), one specialty (e.g. molecular genetics, cytogenetics.), one objective (e.g. targeted mutation analysis, sequence analysis of the entire coding region, methylation analysis.), and one technique (e.g. Sanger sequencing, NGS sequencing, MLPA based techniques). This new model allows for a more precise and user-friendly search for a test or laboratory via the Orphanet website. One significant improvement is that results can be filtered using this newly registered data, as well as by the country of the laboratory and/or data concerning the quality management (accreditation and participation to EQAs). This new model also allows panels of genes used in tests performed by NGS to be included. In Orphanet, these evolutions concern around 40,000 clinical tests, available for 3,500 rare diseases in 39 countries.

**P19.50****Undiagnosed Diseases Network International (UDNI): an international initiative to foster the translation of research into medical practice**

D. Taruscio<sup>1</sup>, S. C. Groft<sup>2</sup>, H. Cederroth<sup>3</sup>, B. Melegh<sup>4</sup>, P. Lasko<sup>5</sup>, K. Kosaki<sup>6</sup>, G. Baynam<sup>7</sup>, A. McCray<sup>8</sup>, G. Floridia<sup>1</sup>, M. Salvatore<sup>1</sup>, W. A. Gahl<sup>9</sup>;

<sup>1</sup>Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Roma, Italy, <sup>2</sup>National Institutes of Health, United States, Bethesda, MD, United States, <sup>3</sup>Wilhelm Foundation, Brotby, Sweden, <sup>4</sup>Department of Medical Genetics, University of Pecs, Pecs, Hungary,

<sup>5</sup>McGill University, Montreal, QC, Canada, <sup>6</sup>Keio University School of Medicine, Tokyo, Japan, <sup>7</sup>Princess Margaret and King Edward Memorial Hospitals, Perth, Australia,

<sup>8</sup>Harvard Medical School, Boston, MA, United States, <sup>9</sup>National Institutes of Health, Bethesda, MD, United States.

Undiagnosed diseases are a global health issue, calling for an international scientific and healthcare effort. In 2008, the National Institutes of Health's (NIH) Undiagnosed Diseases Program (UDP) was initiated to provide diagnoses for individuals who had long sought one without success. Moreover, as a result of two international conferences (Rome 2014 and Budapest 2015), the Undiagnosed Diseases Network International (UDNI) was established, modeled in part after the NIH UDP and the recently formed US-wide Undiagnosed Diseases Network (UDN). The UDNI has published a consensus framework of principles, best practices and governance; the Board of Directors reflects its international character, as it includes experts from Australia, Canada, Hungary, Italy, Japan and the USA. The UDNI involves centers with internationally recognized expertise, and its scientific resources and know-how aim to fill the knowledge gaps that impede diagnosis. Consequently, the UDNI fosters the translation of research into medical practice. Active patient involvement is critical; the Patient Advisory Group is expected to play an increasing role in UDNI activities. After the UDNI launch (2015), several countries have activated national Networks (e.g., Australia, Italy, Au-

stria, Japan) operating in the framework of UDNI and in collaboration with other UDNI members, who convened in Vienna in February of 2016 to plan for data sharing. All information for physicians and patients is available at the UDNI website(<http://www.udninternational.org>)

## P19.51

### Telephone Genetic Counselling: A service evaluation of patient and clinician experiences and impact on service delivery in cancer genetics

R. K. Jassi, C. Patch, A. Srinivas, A. Shaw, A. Kulkarni, D. Ruddy, L. Izatt, V. Tripathi; Guy's Hospital, London, United Kingdom.

**Introduction:** The Guy's cancer genetics service covers a population of 5 million. There has been a steady increase in referrals, clinical queries from secondary care and a demand to increase patient accessibility in our peripheral locations. This prompted us to revisit our current service delivery model. In order to service this need, a model of weekly telephone clinics was initiated as part of a pilot project at Guy's Hospital. Prior to this pilot, all cancer referrals were offered face-to-face clinic appointments. A total of 125 telephone clinic appointments were conducted with patients referred to our cancer service over a six month period.

**Aims:** The aims of the telephone clinics were:

- Manage service demand
- Increase capacity for appointments within same resources
- Increase patient accessibility in wide geographical areas
- Reassure families not at increased familial cancer risk
- Collect and clarify further information required to complete the cancer risk assessment
- Provide fast access to information and advice for those not requiring face to face appointments

**Methods:** Feedback questionnaires were sent to the Cancer Genetics clinicians and patients that participated in each telephone appointment. Reasons for offering or choosing a telephone appointment, ease of understanding and delivering information, and clinician and patient satisfaction were measured and correlated.

**Results and Conclusion:** The findings of this service evaluation as well as its impact on patient and clinician satisfaction will be presented. This evaluation will inform the appropriate use of telephone clinics as an integrated part of the Cancer Genetics Service.

## P19.52

### Why is the teratological counseling necessary?

M. Seven<sup>1</sup>, S. Dasdemir<sup>1</sup>, H. Ulucan<sup>1</sup>, M. B. Duz<sup>1</sup>, E. Kirat<sup>1</sup>, U. Gümüş<sup>1</sup>, M. Demir<sup>2</sup>;

<sup>1</sup>Istanbul University, Cerrahpaşa Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkey, <sup>2</sup>Istanbul University, Cerrahpaşa Faculty of Medicine, Department of Nuclear Medicine, Istanbul, Turkey.

Women exposed radiation without awareness during their pregnancies have often deep concern about possibility of having baby with the congenital anomalies. This situation leads families to make the decision for termination. In this study, the risk of the congenital anomalies due to radiation exposure was calculated and the importance of „teratological counseling“ was demonstrated to avoid unnecessary terminations. 252 pregnant women with the history of radiation exposure enrolled in the study (mean age  $\pm$  S.D.:  $31.40 \pm 5.70$  years, age range: 19-47), gestational ages at admission to the clinic were 4.7-25.2 weeks ( $10.0 \pm 3.96$ ), radiation exposure weeks were 0.3-22.6 weeks ( $4.58 \pm 3.10$ ) and fetal absorbed radiation dose ranged from 0.01 to 10.30 rad ( $0.72 \pm 1.45$ ). 228 of 252 (% 90.47) pregnancies gave birth to healthy children. 3 were terminated for medical purposes while 8 were resulted as spontaneous abortion. We could not reach 6 women. 141 of 252 (% 55.95) pregnant women were suggested to terminate their pregnancy before teratological counseling. After teratological counseling, only 8 of those women preferred to terminate their pregnancy, whereas remaining 132 pregnancies decided to continue the pregnancy and delivered healthy children. Therefore, teratological counseling has provided % 94.3 success of pregnant who had the decision termination thanks to change their mind to continue their pregnancy after teratological counseling. In conclusion, we suggest that teratological counseling is necessary to reduce the anxiety of families and it is the most effective method to prevent unnecessary pregnancy terminations.

## P19.53

### Training the next generation of clinical scientists

M. Cornell<sup>1</sup>, J. Taylor<sup>2</sup>, A. Davies<sup>1</sup>, A. Brass<sup>1</sup>;

<sup>1</sup>University of Manchester, Manchester, United Kingdom, <sup>2</sup>HMDs, Leeds Cancer Centre, Leeds, United Kingdom.

Next generation sequencing (NGS) techniques have the potential to revo-

lutionise diagnostic techniques across healthcare. Along with the development of new whole genome and whole exome analysis for germline variants, stratified treatments for cancers and new preimplantation screening strategies are becoming possible.

In order to translate these advances into real patient benefit, analysis of NGS data needs to be undertaken by highly trained clinical bioinformaticians, of which currently there are acute global shortages.

In the UK, the National Health Service launched Modernising Scientific Careers and has begun the Scientist Training Programme (STP), recruiting students into training programs with specialisms including Clinical Bioinformatics for Genomics, Health Informatics and Physical Sciences.

The University of Manchester has been involved in delivering bioinformatics training to clinical scientists. This has included the development of NGS analysis pipelines and tools for interpreting the effects of genetic variants as well as programming and database development. The course is in its third year and the first cohort of students are approaching graduation.

In this presentation we discuss the development and delivery of training materials, the response of students and mechanisms for delivering further training beyond the UK health service.

## P20 Psychological/Ethical/legal issues

### P20.01

#### Direct-to-consumer advertising of genetic tests: legitimate or not?

L. Kalokairinou<sup>1</sup>, H. C. Howard<sup>2</sup>, P. Borry<sup>1</sup>;

<sup>1</sup>KU Leuven, Leuven, Belgium, <sup>2</sup>Uppsala University, Uppsala, Sweden.

During the past decade, the advertising of direct-to-consumer (DTC) genetic tests (GT) has provoked criticism over its potential adverse impact to public health. In October 2013, the European Parliament suggested in its proposal for Regulation of in vitro diagnostic medical devices that DTC advertising of GT with both direct and indirect medical purpose should be banned. While the proposed Regulation is currently under discussion among the European institutions, this work aims to evaluate the proportionality of forbidding DTC advertising for a broad range of GT. To this end, an overview is provided of the various ways genetic tests have been advertised over the past years and the different ethical issues that have arisen from advertisements. Subsequently, this work examines the laws currently regulating the advertising of GT both at the EU and at the Member States level. Finally, the proportionality of the ban is discussed, drawing a parallel with the discussion regarding the ban on DTC advertising of prescription drugs in Europe. As a conclusion, it is argued that it might be more proportionate to only implement a ban on DTC advertising of GT that may have a direct impact on consumers' health, and impose rigorous rules on DTC advertising for the rest of health-related genetic tests. Such regulation should ensure that promotional claims are substantiated and the benefits, risks and limitations, as well as the target audience of GT are clearly presented.

### P20.02

#### Obstetricians' views on ethics of amniocentesis and its implications on NIPT: discourse analysis from 1969 to 1978 in Japan

M. Sato, K. Muto;

the University of Tokyo, Tokyo, Japan.

[Background] Abortion is prohibited by Japanese criminal law but is legally justifiable when maternal health is threatened. In the 1970s, amniocentesis was introduced across Japan, but was harshly criticized owing to its eugenic and paternalistic implications. The medical society is believed to have changed its outlook towards amniocentesis since that time, placing greater importance on „parental autonomy.“ However, this view has not been verified from a bioethical perspective. We aimed to determine the origin of Japanese „autonomy“ with respect to amniocentesis by analyzing obstetricians' discourses.

[Methods] The authors surveyed three representative journals of obstetrics and selected articles that mention ethical issues pertaining to amniocentesis. Thirty-two articles were selected and categorized based on their ethical references. Next, we discussed the implications of the noninvasive prenatal testing (NIPT) guidelines drawn by the Japan Society of Obstetrics and Gynecology (JSOG).

[Results] Since 1975, reports criticizing selective abortions owing to amniocentesis increased, corresponding with the social attack on the implementation of the prenatal test. Simultaneously, instances emphasizing parental autonomy have increased as well. However, amniocentesis was generally viewed as an inadequate screening test.

[Discussion] Our work suggests that Japanese „autonomy“ with respect to amniocentesis was first discussed by professionals defending amniocentesis against social criticism, which considered it paternalistic. Since its introduction, doctors have observed that amniocentesis should not be applied to all pregnant women. The JSOG guidelines on NIPT succeeded to this approach and additionally guarantee genetic counseling. Therefore, the criticisms regarding the link between amniocentesis and disability discrimination remain to be appropriately answered.

## P20.04

### Living at-Risk for Hereditary Breast and Ovarian Cancer: A Qualitative Study of Concerns and Fears of Genetic Discrimination in Flanders

*A. Wauters, I. Van Hoyweghen;  
KU Leuven, Leuven, Belgium.*

The idea of differential treatment based on personal genetic information has caused a great deal of concern in society and the threat of 'genetic discrimination' has lead to the development of regulations to protect against this in many countries. However, despite these regulatory efforts, people still seem to hold concerns about becoming victims of genetic discrimination. This study aimed to explore whether concerns relating to genetic discrimination are present in Flanders, the origins of these fears, and which coping strategies people employ.

Semi-structured interviews were conducted with 30 carriers of a BRCA1 or BRCA2 mutation, recruited through a Flemish BRCA self-help group. Many participants did not express concerns about genetic discrimination, believing that being at risk for breast- and ovarian cancer would not be considered as a valid basis for discrimination. These participants had never experienced it personally, nor seen it happening to others. However, other participants did hold concerns about either direct or indirect genetic discrimination. They mentioned a range of different contexts in which they felt discrimination could occur, such as insurance and employment, where their concerns originated from seeing others experience discrimination, although genetic information was not the basis for this. They also discussed discrimination in relationships, holding concerns that new partners would not cope with a future overshadowed by the risk of having cancer.

These findings show that despite Belgium's legislative effort to prohibit the use of genetic information, some BRCA mutation carriers are still concerned to become the victim of discrimination.

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## P20.05

### Physicians' messages to unaffected women with BRCA1 and BRCA2 mutations about risk management behaviour. A Swiss qualitative study of patients' experiences

*M. Caiata-Zufferey<sup>1</sup>, V. Cina<sup>2</sup>, O. Pagan<sup>3</sup>, V. Membrez<sup>4</sup>, M. Taborelli<sup>5</sup>, S. Unger<sup>2</sup>, A. Murphy<sup>6</sup>, C. Monnerat<sup>6</sup>, P. Chappuis<sup>7</sup>;  
<sup>1</sup>University of applied sciences of Southern Switzerland, Lugano-Manno, Switzerland,  
<sup>2</sup>Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, <sup>3</sup>Oncogenetics and Breast Unit, Institute of Oncology of Southern Switzerland, Lugano-Viganello, Switzerland, <sup>4</sup>Division of Medical Genetics, Institut Central, Hôpital du Valais, Sion, Switzerland, <sup>5</sup>Oncogenetics and Cancer Prevention Unit, Division of Oncology, University Hospitals of Geneva, Genève, Switzerland, <sup>6</sup>Service of Oncology, Hôpital du Jura, Delémont, Switzerland, <sup>7</sup>Division of Genetic Medicine, University Hospitals of Geneva, Genève, Switzerland.*

**Introduction:** Women with BRCA1 and BRCA2 mutations are at increased risk of breast and ovarian cancer. Managing this risk requires a multidisciplinary approach. However, the literature suggests that healthcare professionals do not always have similar views of genetic risk and its management. This paper explores the messages that physicians convey, consciously or unconsciously, to unaffected genetically at-risk women and the consequences of these messages, based on the experience of the women themselves.

**Material and methods:** Using a grounded theory design, retrospective biographical interviews were conducted with 32 unaffected women with BRCA1 and BRCA2 mutations, who had presymptomatic testing at least three years previously. Data were analyzed with the constant comparative method using ATLAS.ti software.

**Results:** Physicians convey three different messages during the consultation: a normative message (at-risk women are required to take responsibility for their health and to adopt risk-management behaviour); a self-confronting message (at-risk women are invited to question what is essential to them and to act accordingly); a minimizing message (at-risk women are considered as "second-class patients" compared to patients who have presented cancer). Being simultaneously exposed to these conflicting messages, genetically at-risk women experience a strong sense of disorientation and find it difficult to know how to act regarding their risk.

**Conclusions:** There is an urgent need to make physicians aware of the messages they convey to unaffected genetically at-risk women and to promote a shared representation of this condition among healthcare professionals. Study supported by the Swiss National Science Foundation (grant number PZ00P1\_132633 / 1)

## P20.06

### The role and functioning of data access committees: Opinions and experiences of data access committee members and experts

*M. Shabani, P. Borry;  
KU Leuven, Leuven, Belgium.*

**Purpose:** Genomic researchers benefit from broad access to large and information-rich datasets. Data Access Committees (DACS) are established as a governance mechanism to assess data access requests for the purpose of approval or disapproval. This paper explores the perspectives of DAC members and experts on components of access review and functionality of DACs in handling data access requests in accord with the genomic data sharing goals.

**Methods:** Semi-structured interviews were conducted with 16 DAC members and 4 experts in the field, from a diverse international and professional background. Data was analyzed using content analysis methodologies.

**Results:** The interviewees indicated the access review mainly comprises of assessing the ethical footings and the scientific merits of the proposed uses. However, they were ambivalent about the scope and rigor of such review by DACs and the adequacy of available tools and mechanisms to achieve the goals of review. In addition, qualification of the users was subject to scrutiny of DACs. Given the fragmented or poorly delineated qualification criteria at times, the interviewees underscored the significance of adopting standard ways in order to streamline the procedure.

**Discussion and Conclusion:** DACs would benefit from international and institutional policies and guidelines delineating various aspects of access review, including the user's qualification criteria and the sanctions against the violations of policies. Furthermore, the current components of access review should be revisited to reassure the complexity of the access review corresponds to the concerns associated with data sharing and facilitates responsible data use.

## P20.07

### Ethical considerations in sharing genomic data of patients' relatives in cancer research

*K. Takashima<sup>1</sup>, S. Mori<sup>2</sup>, H. Mano<sup>1</sup>, T. Noda<sup>2</sup>, K. Muto<sup>1</sup>;*

*<sup>1</sup>The University of Tokyo, Tokyo, Japan, <sup>2</sup>The Cancer Institute of the Japanese Foundation of Cancer Research, Tokyo, Japan.*

**Background:** Sharing study data with other researchers in repositories or databases will promote scientific progress. In cancer genomic research, not only family history information, but also germline sequence data, including genomic data from patients' relatives, is essential. Sharing these relatives' data requires careful ethical consideration, because future studies may reveal that they present with known or unknown pathogenic variants. Moreover, when the total number of families is small in a given study, the risk of re-identifying family members increases. However, to date, there is no regulation especially for sharing relatives' data.

**Methods:** We investigated the editorial and publishing policies of major scientific journals and the International Committee of Medical Journal Editors on availability of data and protection of research participants. After reviewing all policies, we developed a proactive policy for protection of participants on what researchers should do when they publicly deposit and share relatives' data in their study.

**Results:** Policies on data availability are classified in two categories; submission to a community-endorsed public repository is mandatory and the accession numbers must be provided in the paper, or deposition of sequence data in a repository is encouraged but not required. While most policies have provisions for patient/participant consent and privacy protection, there is no specific rule for relatives' data.

**Conclusions:** We propose recommendations for researchers on how to share genomic data of patients' relatives.

**Grants:** This study was supported by Japan Agency for Medical Research and Development's Project for Development of Innovative Research on Cancer Therapeutics (P-DIRECT).

## P20.08

### Secondary findings on diagnostic exome sequencing: Patient preferences and detection rates based on 1500 DES samples tested at a single clinical laboratory in the United States

C. M. Osborne, L. Shahmirzadi, Z. Powis, C. L. Alamillo, K. F. Hagman, S. Tang;  
Ambry Genetics, Aliso Viejo, CA, United States.

**Introduction:** Diagnostic exome sequencing (DES) involves the simultaneous analysis of virtually all exonic and flanking intronic sequences. Consequently, DES may result in the identification of secondary findings (SF), which are incidental deleterious variants unrelated to the testing indication. In 2013, the American College of Medical Genetics and Genomics (ACMG) issued recommendations pertaining to the reporting of disease-causing mutations within 56 genes identified incidentally during exome or genome analysis.

**Methods:** We examined the evolution of SF test offerings within a single clinical laboratory in the United States from 2011 to 2015. We also performed a retrospective analysis of the patient preferences and positive rates of SF from the ACMG recommended 56 and/or expanded gene lists among the first 1500 patients who underwent DES through our clinical laboratory.

**Results:** Of 1500 DES cases, 437 (29.1%) were ordered before ACMG published recommendations, and 1063 (70.9%) were issued after. Overall, 1361 of 1500 (90.7%) patients requested at least some SF results, and 451 (30.1%) of these cases requested results from more than the ACMG recommended gene list. The majority of cases (1214/1361; 89.2%) had no reported SF, and 47 (3.45%) of patients had at least one reported SF from among the ACMG recommended list of 56 genes.

**Conclusions:** Based on our experience, most patients proceeding with DES elected to receive at least some SF results. Almost 90% of these cases were negative. A continued evolution in the reporting of SF is anticipated as DES becomes increasingly utilized, and as disease-gene relationships are better elucidated.

## P20.09

### Current state and ELSI of DTC genetic testing in minors or fetuses in Japan

N. Ohashi;  
Osaka University, Suita, Japan.

**Introduction:** In addition to uses of genetic testing in clinical and research settings in Japan, direct-to-consumer (DTC) genetic testing services are becoming more common due to the spread on the internet. However, genetic information has Ethical, Legal and Social Issues (ELSI) because of its particularity. Some of those issues occur or become more complicated when the subjects are minors or fetuses who have no or insufficient competency for giving consent. In this presentation I would like to discuss the current state of these ELSI in Japan and consider some solutions.

**Methods:** Investigation and analysis of various research papers, guidelines, and legal regulations at home and abroad

**Results:** While ethical guidelines formulated by related academic societies have no legally enforceable powers, these guidelines have a certain degree of binding effect on their members in the research and clinical fields. However, the industry sector of DTC genetic testing services relies on self-regulation, which is largely ineffective. As a result, there is a great deal of variation in the quality of companies' tests, as well as the ethical standards under which these companies operate.

**Conclusions:** In cases where minors or fetuses are subjects, especially in DNA paternity tests without consent from minors or their mothers and prenatal DNA paternity tests, these tests can seriously harm minors and fetuses—sometimes even causing abortion in the case of fetuses. Regulations—including legal ones—should be made in accordance with the type of genetic testing being conducted.

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## P20.10

### Medical staff approach towards undergoing deep genome sequencing as part of their medical training

A. Harari-shaham<sup>1</sup>, H. Doron<sup>2</sup>, G. Larom-Khan<sup>1</sup>, A. Peleg<sup>1</sup>;  
<sup>1</sup>Carmel medical center, Haifa, Israel, <sup>2</sup>Tel hai College, Upper Galilee, Israel.

**Introduction:** Clinical use of new genetic technologies such as Exome/genome sequencing technologies (E/GST) introduces the medical staff to dilemmas regarding professional, ethical and emotional issues. E/GST might provide information regarding cureless diseases, late onset diseases, risk factors for various medical conditions and variants of unknown significance. Furthermore, such information may affect not only the patient, but also his immediate and extended family. As the use of these technologies expands,

clinicians should be familiar with these professional and psychological issues. A questionnaire survey was conducted among medical staff, examining their approaches and attitudes towards undergoing self-wide genome sequencing as part of routine medical training.

**Materials and methods:** Socioeconomic, cognitive dimension (positions and perceptions), and personality traits questionnaire. Population study: Medical Doctors, nurses, genetic counsellors and medical students. Statistical analysis: T-test, Pearson and regression analysis with SPSS software.

**Results:** Medical Doctors and genetic counsellors significantly preferred to receive only information regarding preventable or curable medical conditions.

Medical doctors and genetic counsellors' approaches were that self E/GST does not improve their clinical skills. The Genetic counsellors' approach differs from the other groups. In their opinion, self E/GST will not provide a better understanding of the ramifications on the patients' emotions.

**Conclusions:** The results of this study provide a more thorough understanding of the approaches of medical staff from different fields towards the use of self E/GST. A better understanding could provide practical implications, for developing professional training programs in regards to the rapidly expanding clinical use of these technologies.

## P20.11

### Guidance to families of children with disabilities as a result of birth defects: contribution of Family Quality of Life Model in Colombia (South America)

D. M. Ortiz<sup>1</sup>, Y. J. Ariza-Araujo<sup>1</sup>, A. F. Ramírez-Botero<sup>1</sup>, H. Pachajoa<sup>1,2</sup>;

<sup>1</sup>Universidad Icesi, Cali, Colombia, <sup>2</sup>Fundación Clínica Valle del Lili, Cali, Colombia.

**Introduction:** This study sought to determine the status of family quality of life in children and youth with disability associated to birth defects, who attended the clinical genetics outpatient consultation at a reference center in southwestern Colombia.

**Materials and Methods:** The Family Quality of Life models proposed by the University of Kansas, and The Family Quality of Life Scale (FQOL) adapted for Colombia, were used to evaluate indicators and scale factors. Each factor was rated in terms of importance and satisfaction according to the caretaker. The Map Family Quality of Life (MFQOL) was configured and shows two areas (critical and strong). The information was saved and processed with the Excel software.

**Results:** The MFQOL showed that most of the indicators were located the strong area of the map, with the exception of some related with family resources and support of persons with disabilities (PWD), which were located in the critical area. Through design strategies it was generated a graphic form to summarize the results and present them clearly to families.

**Conclusions:** information about the needs that families PWD regarding the environment in which they operate, is a first step in developing action plans to define a better care and contribute to the social inclusion of people with disabilities.

## P20.12

### Genetic information disclosure to kin: patients' organizations points of view and roles consequences of a new legal framework in France

D. d'Audiffret<sup>1</sup>, S. de Montgolfier<sup>2,3</sup>;

<sup>1</sup>Lipha Université Paris-Est Marne-la-Vallée, Marne la Vallée, France, <sup>2</sup>IRIS (Ehess/Inserm), Paris cedex 13, France, <sup>3</sup>UPEC, Creteil, France.

In case of serious genetic anomaly, the disclosure of this information, when prevention measures or treatments exist, can be relevant for other family members. In an interdisciplinary research project exploring the consequences of the evolution of the legal framework in France: we assess the impact on the healthcare professionals' practices, patients' reactions and related ethical issues. At the ESHG 2015, we presented the results from an online quantitative research survey dedicated to healthcare professionals. Our aim, now is to get a clearer picture of the challenges arising from this issue regarding patients' associations and to ask them: What is the relevance of transmitting this information to families? What are their needs and expectations? What impact on patients' associations roles?

To explore these questions in a context of numerous genetic diseases, we have organised semi-structured interviews (11/2 hour) with ten patients' organization representatives of different genetic diseases. Thus, we have studied if the patients' perception regarding the genetic information disclosure to family members is impacted by the level of expressivity, age of onset, impacts on the quality of life, access to treatment, or access to genetic counselling. We discussed the online survey completed by healthcare professionals with them in order to identify their point of views.

This study allows us to elaborate recommendations to accompany each sta-

keholder in his role to improve care of patients and families.  
Resarch project financed by INCa and Canceropole Ile-de- France: "Family disclosure in human genetics" (subvention 2013-130).

## P20.13

### Return of research results in a large-scale birth cohort study involving children in Japan

I. Ri, K. Muto;  
University of Tokyo, Tokyo, Japan.

**Background:** The Ministry of the Environment in Japan funded the "Japan Environmental Children's Study" (JECS) in 2011 to follow up on about 100,000 sets of parents, and children from the fetal stage to 13 years of age. As JECS has not determined a detailed plan for genome sequencing, it remains an issue as to how to obtain consent from parent participants and assent from child participants. Moreover, return of the individual results of biomonitoring and environmental exposures has been recommended for the community's benefit [Brody et al. 2014]. However, recommendations about the individual results of genome sequencing in pediatric research require a careful consultation process to ensure the best interests of the children are preserved [Sénécal et al. 2015]. The purpose of our research is to consider the conflicts in both perspectives and to clarify future agendas that will be faced by birth cohort studies.

**Methods:** We carried out a literature review, that included consent forms and relevant documents published by JECS and ethical guidelines.

**Results:** The points of controversy were classified mainly into six categories. Some of the recommendations should be assessed at both the individual and community levels: (1) the balance of risk/burdens and benefits, (2) confidentiality and autonomy, (3) risk communications concerning the pertinent results and incidental/secondary findings, (4) informed assent/dissent and consent, (5) uncertainty, and (6) actionability (not only concerning clinical implications but also life more generally).

**Discussions:** It is necessary to investigate participant and community attitudes toward genome sequencing, and how results are communicated.

## P20.14

### Professionals' reactions on indirect disclosure of genetic information to kin: first lessons of the French experience

B. Derbez<sup>1</sup>, S. de Montgolfier<sup>1,2</sup>;  
<sup>1</sup>IRIS (Ehess/Inserm), Paris cedex 13, France, <sup>2</sup>Université Paris Est Créteil, Creteil, France.

French bioethics law of 2011 has had the opportunity to create an original device to face issues of genetic information to kin. According to the law, people who undergo a genetic test have the legal duty to inform their family members in case of serious illness for which measures of care, prevention or genetic counselling are available. In case of difficulty, professionals can directly contact the patient's relatives to inform them about a potential genetic risk. To do it, the professionals need to acquire the consent of the index case and to preserve his/her anonymity. However, how have they grasped this new device ? How is the „indirect way“ practiced in the daily activity of genetic services?

To answer these questions, we propose to explore data of a one-year qualitative fieldwork (observations and interviews) in genetic wards of two hospitals in France. The first was specialized in genetic predispositions to cancers (breast and ovarian mainly) and the second in genetic diseases of red blood cells (sickle cell disease and hemochromatosis in particular).

Firstly, in this presentation, we propose to analyse few cases where „indirect way“ has been set up at the request of patients by asking each other how the procedure was negotiated between the social actors. Secondly, this will lead us to question the relationship of professionals with a device that seems to be at odds with some medical action standards.

Resarch project financed by INCa and the Canceropole Ile-de- France. "Family disclosure in human genetics" (subvention 2013-130)

## P20.15

### Quality of life in adults with Neurofibromatosis 1 in Brazil

D. G. Melo, N. P. Bicudo, B. F. Menezes Neto, L. R. S. de Avó, C. M. R. Germano;  
Department of Medicine, Federal University of São Carlos, São Carlos, São Paulo, Brazil.

**Introduction:** Neurofibromatosis type 1 (NF1) is a neurocutaneous genetic disorder that can be associated with severe complications, and it may shorten patients' lifespan and affect their quality of life negatively. This study aimed to examine quality of life constructs among adults with NF1 in Brazil. **Methods:** It is an exploratory, descriptive and cross-sectional study consisting of two stages, involving thirteen adult patients with NF1, who have a

wide range of severity and visibility of the disease. The first stage was developed using a quantitative methodology, namely the WHO Quality of Life-100 questionnaire; responses for the patients were compared to a matched control group. The second stage comprised clinical-qualitative research whereby patients took part in a semi-structured interview; these data were analyzed using the categorical thematic analysis technique.

**Results:** There were no statistically significant differences in the questionnaire domains between the NF1 patients and the control subjects. Eighteen main themes were extracted from the interviews, showing interference of the NF1 visibility principally in psychological aspects and social relationships. Patients mentioned curiosity about NF1 and confusion about the distinctions between NF1 and contagious diseases, which lead to prejudice. They were concerned about the future and how the disease would develop in themselves and their offspring, and emphasized difficulties acquiring proper healthcare.

**Conclusions:** These findings may help in planning healthcare for Brazilian NF1 patients and improving their quality of life.

This survey was supported by the São Paulo Research Foundation (FAPESP, grant 13/25330-3).

## P20.16

### Boosting medical research innovations at European level thanks to a wider dissemination of patents

F. Le Corre<sup>1</sup>, G. Chassang<sup>2</sup>, M. Roques<sup>1</sup>, E. Rial-Sebag<sup>2</sup>;

<sup>1</sup>Inserm, Toulouse, France, <sup>2</sup>JuristeConsult, Toulouse, France, <sup>3</sup>Inserm, University Toulouse III Paul Sabatier, Toulouse, France.

Patents confer the right to exclude others from making, using, or selling a claimed invention, for a period of 20 years from its filing date. The paradox raised by patents is that they ensure a monopoly to the patent holder on his invention while it might be considered as a brake on innovation because when a patent owner exploits his patent, no-one else can use the technology without asking for a license. Then, if a researcher wants to use several patented technologies for his own project, he will need to negotiate with all patent holders which is considered as time consuming and expensive. A wider dissemination of inventions can be offered while preserving the patent-owners' rights, through a collaborative process known as "patent pool" or "clearing houses" mechanisms.

In this system, a specific scientific domain can be targeted by stakeholders who can decide to propose global governance for the intellectual property rights and for the access to their patents. This collaborative licensing model already exist (e.g. for AIDS), it makes patented technology landscape more transparent and it leads to an easier access to patents and to a significant reduction of transactions costs.

A collaborative model at a European infrastructure level (BBMRI-LPC) could be proposed to favor the transfer of technology for medical research at large. It might be considered as a solution to the roadblocks in accessing to essential technologies by researchers who want to use inventions within their specific area of expertise.

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## P20.17

### The offer of expanded preconception carrier screening to couples: are there differences in views within couples and do views change after discussion?

M. Plantinga<sup>1</sup>, J. Voorwinden<sup>2</sup>, J. Schuurmans<sup>1</sup>, A. Lucassen<sup>3</sup>, A. Buitenhuis<sup>4</sup>, E. Boersma<sup>1</sup>, E. Birnie<sup>1</sup>, M. Verkerk<sup>1</sup>, J. van Langen<sup>1</sup>, A. Ranchor<sup>2</sup>;

<sup>1</sup>UMCG, Department of Genetics, Groningen, Netherlands, <sup>2</sup>UMCG, Health Psychology, Groningen, Netherlands, <sup>3</sup>Faculty of Medicine, Clinical Ethics and Law (CELS), Southampton, United Kingdom, <sup>4</sup>UMCG, Department of Internal Medicine, Groningen, Netherlands.

**Introduction:** Technological developments in genome sequencing allow for rapid and cheap testing of many autosomal recessive diseases concurrently. The University Medical Center Groningen (UMCG) developed a preconception screening (PCS) test which covers 50 serious, early onset, untreatable autosomal recessive diseases. The test is offered and analysed per couple. Results are communicated as 'couple has increased (1:4) risk of having a child with disease X' or 'no increased risk detected for this couple'. We think this is ethically, technically and economically justifiable. However, views of eligible couples, and specifically the extent to which they agree with each other, are not known yet.

**Materials and Methods:** By means of an online survey we explored how couples view the offer of a couple-based PCS test. We examined discrepancies within and between couples (n=117) and before and after (n=84) discussion of test-information.

**Results:** Views differ between couples: 45% of the couples are positive about PCS testing, 10% are negative and 45% are neutral. Of all couples, 30% intend to test, 20% do not and 50% are neutral. Within couples there is large agreement: in 70% of the couples both partners display the same level of attitude and intention. Views change after discussion of test-information. After discussion, respondents displayed a significantly more positive attitude towards PCS testing. Intention also increased, but only in couples with low relation satisfaction.

**Conclusions:** Eligible couples are positive about the offer of a couple-based PCS-test. Views differ between but not within couples and become more positive after discussion of test-information.

## P20.19

### Analysis of a new national initiative for the implementation of genome medicine in Japan

**J. Minari<sup>1</sup>, K. Kato<sup>1,2</sup>;**

<sup>1</sup>Department of Biomedical Ethics and Public Policy, Graduate School of Medicine, Osaka University, Osaka, Japan, <sup>2</sup>Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan.

Recently, leading programs such as the Precision Medicine Initiative in the US and Genomics England in the UK have promoted the implementation of genome medicine. These national-level initiatives aim to demonstrate the complex relationship between genetic, lifestyle and environmental factors regarding the development of disease, foreseeing individualized diagnosis, prevention or treatment of disease.

In Japan, a new policy movement to implement genome medicine has recently started. In 2012, Japan established the Headquarters of Healthcare Policy (HHP) to promote healthcare innovation. The committee charge of 'genome medicine', positioned under the HHP, has released a new policy for the achievement of genome medicine, and showed the approach of research development as well as of improving the environment for genome medicine. In addition to these movements, the Act on the Protection of Personal Information was revised in 2015, so that the ethical and legal regulation regarding genome information are currently being reviewed.

In this study, we have examined the current policy movements, including ethical considerations, related to genome medicine in Japan. We have clarified the drafting process and major points of the new policy, and suggested some of the main challenges and opportunities in its implementation. One of the main findings is that the latest policy has significantly emphasized role-sharing arrangements and organic co-operation primarily between cabinet secretariat, ministries and funding agencies. This study represents a valuable opportunity to share an experience regarding policy developments in Japan with other countries and to explore and develop new methods of policy making.

## P20.21

### Rights and responsibility in genomic decision-making: a systematic review of qualitative and quantitative studies exploring stakeholder views on secondary findings in genomic sequencing

**M. P. Mackley, B. Fletcher, M. Parker, H. Watkins, E. Ormondroyd;**  
University of Oxford, Oxford, United Kingdom.

**Purpose:** The return of secondary findings (SF) in whole-genome and -exome sequencing (WGS/WES) is a subject of much debate. As WGS/WES moves into routine clinical practice, it is timely to review data that might inform this debate in the development of policies that maximize benefit for participants.

**Methods:** We systematically searched 6 electronic databases for qualitative and quantitative studies that explored stakeholder views on SF in WGS/WES, in clinical and research settings. Framework analysis was undertaken to identify major themes, and assess the extent to which there was sufficient evidence base for informing policy on SF in WGS/WES.

**Results:** Thirty-two articles reporting views of over 11,000 stakeholders were found to meet inclusion/exclusion criteria. Stakeholders were broadly supportive of returning life-threatening and treatable SF, but varied in views on other types of SF. Largely, views of sequencing providers focused on the idea of 'responsibility' and non-providers focused on 'rights'.

**Conclusions:** Stakeholders agreed on importance of patient autonomy and generally supported a model of shared responsibility in SF management. Professionals, therefore, need to balance their responsibility to care for participants with sharing decision-making. To do so, professionals need to help participants exercise their rights in an informed manner. This has implications for pre-test discussions and informed consent, as technical knowledge was not found to lead to more informed decision-making. Further research into what best informs decisions around SF is, therefore, essential. As 70% of the included studies were American, it is crucial that research gathering European and other international perspectives be conducted.

## P20.22

### Disclosing genetic information to family members: a comparative-law study of the legal regimes applicable to patients' and health professionals' liability

**C. Farnos<sup>1</sup>, L. Battistuzzi<sup>2</sup>, Á. Mendes<sup>3,4</sup>, P. Borry<sup>5</sup>, H. Nys<sup>6</sup>, M. Rothstein<sup>6</sup>, C. Mitchell<sup>7</sup>, P. Nicolas<sup>8</sup>, D. Sprumont<sup>9</sup>, E. Rial-Sebbag<sup>1</sup>;**

<sup>1</sup>Inserm UMR 1027, University of Toulouse III – Paul Sabatier, Faculty of medicine, Toulouse, France, <sup>2</sup>Dept. of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy, <sup>3</sup>IS – Instituto de Investigação e Inovação em Saúde, Univ. Porto, Porto, Portugal, <sup>4</sup>UnIGENE and Centre for Predictive and Preventive Genetics (CGPP), IBMC – Institute for Molecular and Cell Biology, Univ. Porto, Porto, Portugal, <sup>5</sup>Centre for Biomedical Ethics and Law, University of Leuven, Leuven, Belgium, <sup>6</sup>Institute for Bioethics, Health Policy and Law, University of Louisville School of Medicine, Louisville, KY, United States, <sup>7</sup>HeLEX - Centre for Health, Law and Emerging Technologies, University of Oxford, Oxford, United Kingdom, <sup>8</sup>Interuniversity Chair in Law and The Human Genome, University of the Basque Country, Bilbao, Spain, <sup>9</sup>Institut de droit de la santé, Université de Neuchâtel, Neuchâtel, Switzerland.

Genetic information is often considered as 'exceptional' health information because of its personal, predictive and familial dimension. Indeed, the diagnosis of a serious genetic anomaly in a patient can be clinically relevant for other family members when preventive measures or treatment exists. However, the transmission of such confidential medical information to at-risk relatives questions some fundamental principles of health law, such as medical secrecy, the right to privacy, the right to health information, or the right not to know.

In France, the legislator introduced a specific system for disclosing genetic information to family members in 2004, revised in 2011 by the last Bioethics law. The patient has now a legal duty to inform his relatives about genetic risks when it is relevant for their health. This information can be delivered directly by the patient, or indirectly through a specific procedure involving the prescribing medical doctor. Although the French legislator tried to conciliate the various rights raised, the patient and the prescribing doctor are in charge of many procedural obligations, which have significant implications in terms of liability. Indeed, if the patient does not inform his relatives, he could be held liable under civil law.

A comparative study of eight countries (Italy, Portugal, Belgium, USA, UK, Spain, Switzerland and France) on the disclosure of genetic information to family members will be carried out, with a focus on the liability regimes applicable to patients and health professionals, and on their judicial consequences.

## P20.23

### Citizen's Health through public-private Initiatives: Public health, Market and Ethical perspectives

**P. Borry<sup>1</sup>, I. Budin Ljøsne<sup>2</sup>, H. Beate Bentzen<sup>2</sup>, O. Feeney<sup>3</sup>, H. Howard<sup>4</sup>, M. Loi<sup>5</sup>, D. Mascalzoni<sup>6</sup>, S. Nordal<sup>6</sup>, H. Skirton<sup>7</sup>, S. Sterckx<sup>8</sup>, E. Stupka<sup>9</sup>, H. Felzmann<sup>3</sup>;**

<sup>1</sup>University of Leuven, Leuven, Belgium, <sup>2</sup>University of Oslo, Oslo, Norway, <sup>3</sup>National University of Ireland, Galway, Ireland, <sup>4</sup>University of Uppsala, Uppsala, Sweden, <sup>5</sup>ETH Zurich, Zurich, Switzerland, <sup>6</sup>University of Iceland, Reykjavík, Iceland, <sup>7</sup>University of Plymouth, Plymouth, United Kingdom, <sup>8</sup>Ghent University, Ghent, Belgium, <sup>9</sup>Boehringer Ingelheim, Neu-Ulm, Germany.

Rapid advances in sequencing technologies are accelerating the integration on and use of genomic information in research setting and clinical practice. Moreover, citizens are being confronted with an expansion of genetic testing services available through the internet. Engaging with the generation and interpretation of genetic data represents a tremendous opportunity but also poses new challenges for researchers, healthcare providers, policy makers and society. Existing ethical and regulatory frameworks may not be suitable in dealing with this increasing availability of genomic information. In 2014, the COST Action CHIPME, comprised of members from 27 European countries, began its work to address the need of interdisciplinary reflection on these developments in the European context. CHIPME focuses on the following objectives: (1) Advancing the study of the ethical, legal and social implications of new developments in genetics by building a strong network of early stage and experienced researchers from a wide range of disciplinary backgrounds. (2) Achieving communication between a diverse group of European stakeholders in the field of human genetics, including social science and bioethics experts, genetic researchers, public institutions and companies. (3) Building capacity by facilitating new connections between stakeholders and connecting established scholars with early stage researchers. The Action addresses ethical and regulatory issues with regard to innovations in biobanking, DTC genetic testing, large scale sequencing projects, personalized medicine and participant-led initiatives, and is developing a White Paper addressed to policy makers.

## P20.24

### Transparency and clinical trials in Europe: case study on the incidents in France

**G. Chassang<sup>1</sup>, F. Taboulet<sup>2,3</sup>**

<sup>1</sup>JuristeConsult Ethique et Santé, Toulouse, France, <sup>2</sup>INSERM UMR 1027, Toulouse, France, <sup>3</sup>Université Paul Sabatier Toulouse III, Toulouse, France.

**Background:** Clinical trials are essential activities for scientific and medical progress that involve interventions on human beings which voluntarily participate to biomedical research projects for testing new drugs, medical devices or methods. Biomedical research protocols intrinsically involve some risks for the participants which have to be outweighed by the health/scientific benefits and controlled by researchers and public authorities. Participants are entitled to know about these risks before consenting to the trial and their interests should always prevail on scientific interest.

EU law and bioethical guidelines promote transparency and data sharing principles as obligations for research sponsor/promoters and investigators.

Allowing independent assessment and public oversight of the activities, transparency mechanisms are supporting fairness, safety, precaution, social responsibility and research integrity in innovation. Transparency should find concrete applications through projects' designs and be particularly respected where security problems arise and engage the health of the participants. This poster summarises the key points of transparency implementation, as required by law and good clinical practices and illustrate the needs for transparency in the rare context of risk realization.

**Discussion:** In the light of the inquiry on the drama that occurred in January 2016 with the phase 1 BIOTRIAL clinical trial (EudraCT n° 2015-001799-24) conducted in France that caused the death of an healthy participant and emergency hospitalization of 5 other volunteers, this poster tends to inform and question crisis management from an ethical point of view while the EU Clinical Trial Regulation of 2014 is going to be enforced in the coming years.

## P20.25

### Parent Perspectives on Whole Genome Sequencing for Autism Spectrum Disorder

**M. J. Szego<sup>1,2</sup>, J. A. Anderson<sup>1</sup>, N. Hoang<sup>2</sup>, P. Szatmari<sup>1,2</sup>, S. W. Scherer<sup>1,2</sup>**

<sup>1</sup>University of Toronto, Toronto, ON, Canada, <sup>2</sup>The Hospital for Sick Children, Toronto, ON, Canada.

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions that have a complex genetic etiology. Whole genome sequencing (WGS) is a powerful ASD research tool since it can assay many types of genetic variation in a single test. In recent years, there has been a lot of debate around return of results following genetic testing; however, there is a paucity of empirical data on research subject's experience with WGS, in particular. To elicit and analyze participants' experiences with WGS, we invited parents (and capable children) who were enrolled in an ASD genomic study to participate in semi-structured interviews at two times during the WGS testing cycle: 1) following informed consent, and 2) following return of results. To date, we have interviewed 19 parents at time 1 and 8 parents at time 2. Emerging themes from time 1 interviews include, hope for a genetic diagnosis, hope for treatment, and altruism. Parents also hoped for findings that they could use for planning and prevention purposes, although they expressed concerns over inflicted insight and insurance discrimination. Following return of results at time 2, emerging themes include, the value of learning genetic information, the resolution of blame between biological parents, and disappointment at not receiving treatment-guiding information. Our findings suggest that participants may have different motivations/concerns before and after testing; whereas many begin with altruistic motivations, their desire for actionable results weighs heavily post testing. Though our analysis is provisional, these results suggest that novel strategies for education and informed consent are necessary.

# ELECTRONIC POSTERS

## E-P01 Reproductive Genetics/Prenatal Genetics

### E-P01.03

#### Outcome of aneuploidy screen positive test based on amniocentesis results in 100 Iranian pregnant women

**S. Mesghali, M. Fatahi, F. Harrazizadeh-e-yazdi;**  
Yazd Genetic, Yazd, Iran, Islamic Republic of

**Background:** About half of spontaneous abortions are caused by chromo-

somal abnormalities. Furthermore, diagnosis of chromosomal abnormalities during first and second trimesters of pregnancy can lead to detection of many infirmities and treated abortion.

**Method:** We analyzed 100 pregnant women with evidence of chromosomal aberrations. Among them 83% had positive in screening serum test, 3% with abnormal sonographical soft markers, and 6% with both of serum screening and sonographical soft marker, 7% with past history of chromosomal abnormal child, 1% with maternal chromosomal abnormality. Amniocytes were cultured and then the fetus karyotypes were cytogenetically analyzed.

**Results:** Normal karyotyping was in 80% of fetuses (39% male and 41% female). Minimal change and major chromosomal aberrations were seen in 20% of fetuses, consisting of: 9qh+ (6), 22ps+(1), 21ps+(1), translocation(5,9)(1 case), inversion 9 (2), Down syndrome (47,XX+21)(1 case), Down syndrome with Robertsonian translocation (1), mosaic Turner syndrome(1).

**Conclusion:** Due to diagnosis of many chromosomal abnormalities by non-invasive screening test we can confirm that these screening methods are really useful and highly recommended in prenatal diagnosis.

### E-P01.04

#### Evaluation of amniocentesis decision-making: 4447 patients from a tertiary reference center

**K. M. Erdogan<sup>1</sup>, O. Kandemir<sup>2</sup>, S. Yalvac<sup>2</sup>, S. Esin<sup>2</sup>, F. Alpaslan Pinarli<sup>3</sup>, A. Tukun<sup>4</sup>**

<sup>1</sup>Etilik Zubeyde Hanım Gynecology and Obstetrics Training and Research Hospital

<sup>2</sup>Department of Medical Genetics, Ankara, Turkey, <sup>3</sup>Etilik Zubeyde Hanım Gynecology and Obstetrics Training and Research Hospital Department of Perinatology, Ankara, Turkey,

<sup>4</sup>Diskapi Yıldırım Beyazıt Training and Research Hospital Stem and Genetic Diagnosis Center, Ankara, Turkey, <sup>4</sup>Ankara University Medical School Department of Medical Genetics-Duzen Laboratories Group, Medical Genetics Division, Ankara, Turkey.

Detection of trisomies especially trisomy 21 in the fetus is the major goal of amniocentesis. As this procedure is invasive and has a small risk of miscarriage amniocentesis is usually offered to women with high risk. For the risk estimation of pregnant women, it is important to know the frequency of chromosomal abnormalities according to the different clinical indications. We analyzed retrospectively the cytogenetic results of 4447 amniotic fluid samples referred to our hospital from 2008 to 2012. Chromosome abnormalities were detected in 2.6%. Classical autosomal trisomies were the most frequent ones (45,2%). Positive prenatal screening and advanced maternal age (AMA) were the most common referral reasons with 56,1% and 38,9%. Positive predictive value of ultrasound abnormalities was highest with 5,8% and for increased triple test screening risk and AMA it was 2,3% and 2,7%, respectively. When the karyotype analysis results of amniocentesis according to abnormal and normal karyotype results performed for AMA and increased triple test compared, the difference was statistically not significant ( $p>0.05$ ).

### E-P01.05

#### Aneuploidy findings on two different pregnancy loss materials from the same mother

**I. Buran<sup>1</sup>, E. M. Ay<sup>1</sup>, G. Dogru<sup>1</sup>, O. Izci Ay<sup>1</sup>, U. Karakas<sup>1</sup>, K. Cevik<sup>1</sup>, D. Yuksel<sup>1</sup>, U. Dilek<sup>2</sup>, E. M. Erdal<sup>1</sup>**

<sup>1</sup>Mersin University, Faculty of Medicine, Department of Medical Biology, Mersin, Turkey,

<sup>2</sup>Acibadem University, Faculty of Medicine, Department of Gynecology and Obstetrics, Istanbul, Turkey.

**Introduction:** Early miscarriages are the most common complications of pregnancies and frequency of such cases is about 10-15% of all pregnancies. Endocrinological and anatomical causes, infectious diseases, environmental factors, immunological defects, genetic factors including numerical and structural chromosomal aberrations are the main causes for early miscarriages. The most common karyotypic anomaly in early miscarriages is autosomal trisomy. Chromosomal aberrations play an important role in the etiology of early miscarriages.

**Materials and Methods:** 37 years old pregnant female patient who has a healthy daughter was admitted to Polyclinic of Gynecology and Obstetrics at Faculty of Medicine in Mersin University for the missed abortion. Abortion materials from two separate pregnancies were sent to laboratory at different times. Samples from the first fetus from the 8th week of the pregnancy and the samples from the second fetus from the 9th week of pregnancy were cultured and metaphase plates are obtained using GTG banding technique. 15 metaphase plates from the first fetus and 20 from the second fetus were cytogenetically examined.

**Results:** As the result of cytogenetic analysis, 15 cells obtained from the fetal tissue of the first pregnancy showed 47,XX+12 karyotype and 20 cells from the second pregnancy showed 48,XY+16,+22 karyotype.

**Conclusions:** Aneuploidy is the primary cause of the first trimester miscarriages. Cytogenetic studies showed that aneuploidy level can be at 50-80% in certain populations. While autosomal trisomy is the most common karyotypic anomaly among these, polyploidy, sex chromosome anomalies and structural rearrangement can also be found at high rates in spontaneous miscarriages.

#### E-P01.06

#### Usefulness of array-CGH technique for genetic counseling regarding Y;autosome translocations in prenatal diagnosis

*A. Pavel<sup>1,2</sup>, L. Dimos<sup>1</sup>, M. Necula<sup>1</sup>, I. Pavel<sup>1</sup>, F. Nedelea<sup>1,3</sup>, V. Plaiasu<sup>1,4</sup>, R. M. Simion<sup>1</sup>, D. Stamboiu<sup>1</sup>;*

<sup>1</sup>*Cytogenomic Medical Laboratory, Bucharest, Romania, <sup>2</sup>"Stefan S. Nicolau" Institute of Virology, Romanian Academy, Bucharest, Romania, <sup>3</sup>Filantrópia Hospital, Bucharest, Romania, <sup>4</sup>Mother and Child Care Institute "Alfred Ruseșcu", Bucharest, Romania.*

**Introduction:** Structural chromosomal abnormalities, balanced or unbalanced, may be inherited from a carrier parent or may occur as de novo rearrangements. When the abnormality occurs as a de novo event, the risk for genetic disease is increased, even when the rearrangement appears balanced. This may result from either submicroscopic deletions or duplications at the breakpoints. Like any other chromosome, Y chromosome can be translocated onto an autosome, in a balanced or unbalanced way, with a low incidence.

**Materials and methods:** We report two prenatal cases with a de novo Y;autosome translocation resulting in submicroscopic deletions. The cases were analysed by conventional karyotype using standard chromosome preparation technique. The investigation continued with targeted array-CGH technique, using BAC clones for the identification of potential submicroscopic imbalances. All results were confirmed by FISH analysis using specific probes.

**Results:** Conventional karyotype revealed abnormal karyotypes for both cases with 45 chromosomes and a translocation of the entire Y, (or at least the entire long arm) to an acrocentric autosome, chromosome 13 and chromosome 15 respectively. Array-CGH detected genomic imbalances, more precisely, a deletion of 305.6Kb on Yp of the SRY gene for the first case and a deletion of 6.5Mb on chromosome 15q of the Prader Willi/Angelman region for the second case. Both array-CGH results were confirmed by FISH.

**Conclusions:** Array-CGH method increases the detection of chromosomal abnormalities for high risk pregnancies and allows for more accurate genetic counseling, recurrent risk assessment and for more informed reproductive decision making.

#### E-P01.12

#### Mutation analysis of Tudor Domain in TDRD5 gene and association study of rs508485(HIW12) and rs11703684(HIW13) polymorphisms in Iranian azoospermic men

*Z. Kamalian, R. Mirfakhraie, S. Pooryamanesh;*

*Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.*

**Introduction:** Azoospermia, the most common reason for male infertility, is caused by spermatogenetic failure. Recently, the role of piRNA pathway has been approved in spermatogenesis. The purpose of the present study was to mutation analysis of the Tudor domain in TDRD5 gene and also association study of rs508485(T>C) in HIW12 and rs11703684(C>T) in HIW13 genes in Iranian men with idiopathic non-obstructive azoospermia.

**Materials and methods:** Genomic DNA was extracted from blood samples obtained from 108 azoospermia samples and 100 healthy controls. Probable mutations in exons 9 and 10 of TDRD5 gene were screened using multi-temperature single strand conformation polymorphism (MSSCP) technique. Genotyping was performed using Tetra-ARMS-PCR for rs508485(T>C) and rs11703684(C>T) polymorphisms.

**Results:** Significant difference in distribution of rs508485 genotypes was found in azoospermia cases in comparison to controls, with P-value of 0.035 and odds ratio equal to 2.00 (95% CI: 1.04-3.86). No mutation was detected in Tudor domain of the TDRD5 gene in the patients.

**Conclusion:** We provide, for the first time, evidence for association between genetic variation the genes involved in the piRNA pathway and azoospermia in Iranian patients. Therefore, piRNA genes variants can be considered as risk factors for male infertility. Further studies will be required to validate the significance of the studied genetic variation in diverse ethnic populations.

#### E-P01.13

#### A novel TAZ gene mutation detected prenatally in a family with Barth syndrome

*E. Benušienė<sup>1,2</sup>, M. Rachlevičiūtė<sup>1</sup>, A. Morkūniene<sup>1,2</sup>, L. Ambrozaitytė<sup>1,2</sup>, A. Utkus<sup>1,2</sup>;*

<sup>1</sup>*Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Centre for Medical Genetics, Vilnius University Hospital Santariskių Klinikos, Vilnius, Lithuania.*

Barth syndrome (OMIM 302060) is a rare X-linked disease characterized by dilated cardiomyopathy, proximal skeletal myopathy and cyclic neutropenia. Barth syndrome is caused by various mutations in the tafazzin (TAZ) gene which causes abnormalities of cardiolipin, an essential mitochondrial phospholipid.

A 22-year-old woman, G1 P0, was referred to our unit at 13 weeks of gestation for genetic counselling because of familial history of cardiomyopathy. Her first brother died at 6 months of age due to cardiac failure. The second brother on the 10th day of his life was diagnosed with cardiac insufficiency. At 1 year and 4 months of age the boy was diagnosed with growth retardation, hypotonia, cardiac insufficiency, hypoglycemia and neutropenia. Biochemical findings were excess of 3-methylglutaric and 3-methylglutaconic acid in urine and low free carnitine in blood. Barth syndrome was admitted based on clinical, biochemical findings and family history. The boy died of cardiopulmonary insufficiency at 11 years of age.

The first trimester ultrasound scan determined the male fetus and chorionic villus sampling was performed. DNA testing revealed a novel TAZ gene mutation c.285-1G>C. The same heterozygous mutation was detected in the woman - carrier status was confirmed. Other members of the family were also tested. Results of the mutational analysis offers the possibility of prenatal genetic counselling and preimplantation genetic diagnosis for the family.

#### E-P01.16

#### Assisted reproductive technologies for infertile couples with chromosome abnormalities - 6-years' experience results of "Center of Reproductive Medicine"

*S. Zhukovskaya<sup>1</sup>, N. Rumiantseva<sup>1</sup>, S. J. Zhukovskaya<sup>2</sup>;*

<sup>1</sup>*Center of Reproductive Medicine, Minsk, Belarus, <sup>2</sup>Belarusian State Medical University, Minsk, Belarus.*

**Introduction:** Chromosome abnormalities cause infertility, reproductive failures, birth of affected offsprings. **Materials and methods:** We present cytogenetic data and ART results of infertile patients that underwent treatment in 2010-2015. **Results:** 87 patients (1.5%, 60 males, 27 females) with chromosome abnormalities found among 5846 examined people. Balanced rearrangements diagnosed in 51 cases (58.6%): 22 (43.1%) reciprocal translocations (including X;autosome), 9 (17.6%) Robertsonian translocations, 18 (35.2%) inversions (including 4 paracentric), 2 (3.9%) insertions. Carriers showed normal gender determination, mental, growth development. Men presented spermatogenesis impairment, testicular hypoplasia, varicocele/ cysts. Females presented hormonal imbalance, menstrual dysfunction, ovarian cysts, endometriosis. 36 patients (41.4%) presented unbalanced karyotype including mosaic forms. Among 26 males 20 patients presented numerical gonosome abnormalities, 4 - structural Y-chromosome aberrations; one case 46,XY,r(21)(p11.2;q22.3) and heteromorphisms 46,Y,15q11.2var. Females presented 6 cases of numerical aberrations (monosomy X; trisomy ), 3 cases 47,XX,+mar, one case 46,XX,del(X)(q26). Patients displayed normal mental health, no malformations.

Polysomy X males presented azoospermia, testicular hypoplasia, hormonal imbalance. Monosomy X females had primary amenorrhea. Mosaic or structural gonosome's abnormalities carriers displayed hypogenitalism of various severity. 48 balanced rearrangements carriers (17 females; 31 males) underwent ART (27 IVF, 21 IVF+ICSI). Pregnancy occurred in 23 cases (47.9%). 6 pregnancies resulted in miscarriage, 11 - term delivery, 5 pregnancies are continuing. 13 unbalanced carriers used ART (6 IVF; 7 IVF+ICSI). Pregnancy occurred in 3 cases (23%): 2 miscarriages, 1 continuing pregnancy. 91% pregnant women underwent invasive diagnostics; no foetuses with unbalanced karyotype were detected. **Conclusion:** cytogenetic screening must be recommended to all couples undergoing infertility treatment.

#### E-P01.18

#### Copan human DNA free FLOQSwabs™ stored in eNAT medium are a good buccal collection device for genetic investigations

*A. Squassina, S. Castriciano;*  
*Copan Italia, Brescia, Italy.*

**Introduction:** Genetic diagnostic has increased in the last decade. Buccal swabs are considered an alternative to blood for collecting genetic material,

since are not invasive, well accepted by donor and cost effective. Copan is producing a human DNAfree FLOQSwabs™ line, available dry or associated to a tube of eNAT™, a molecular medium that preserves DNA at RT and inhibits bacterial flora and allows the possibility to perform multiple tests from the same donor. The objective of this study was to evaluate short and long term storage stability of human DNA from buccal swab collected with hDNA free FLOQSwabs™ stored in eNAT medium.

**Methods:** Buccal swabs replicates were collected with FLOQSwabs™ and stored in eNAT medium from 30 donors. Swabs were stored at room and refrigerated temperature for 1, 4, 8 and 12 months. 200  $\mu$ l of each eNAT sample was used to extract Human DNA at each time point using PrepSEQ Express and AutoMate Express, quantified using Quantifiler Trio onto 7500 Real Time PCR machine, and profiled using Identifiler Plus (Life technologies).

**Results:** The buccal swabs in eNAT medium gave an average of 10 ng/ $\mu$ l and good quality DNA. Minimal loss of DNA was observed after 1 and 4 months at room and refrigerated temperature. Longer stability is ongoing.

**Conclusions:** Copan hDNA free FLOQSwabs™ stored in eNAT medium are a good device for buccal swabs collection for human DNA genetic investigations, HLA testing for donor screening, paternity testing, cancer markers, and a diagnostic tool for infectious or hereditary diseases.

#### E-P01.19

#### Comparative Genomic Hybridization for genetic evaluation of early fetal losses

Z. G. Markova<sup>1</sup>, E. V. Musatova<sup>1</sup>, Y. O. Kozlova<sup>1</sup>, N. V. Shilova<sup>1</sup>

*Federal state budgetary institution «Research centre for medical genetics», Moscow, Russian Federation.*

**Introduction:** Spontaneous abortion is one of the most frequent problem of pregnancy.

More than 50% of all spontaneous abortions have abnormal karyotype. Comparative genomic hybridization (CGH) can detect chromosomal abnormalities associated with spontaneous abortions while avoiding the technical problems associated with tissue culture.

**Materials and Methods:** A total of 76 spontaneous abortions of the first trimesters (4 -12 weeks of gestation) were studied by CGH. The additional aneuploidies detected by CGH were all confirmed by targeting FISH with corresponding DNA probes. All samples shown to be chromosomally balanced in CGH underwent interphase FISH to determine ploidy with DNA probes for chromosomes 18, X and Y.

**Results:** The overall rate of detected numerical chromosomal abnormalities was 57%. Trisomies of autosomes were the predominant chromosomal aberrations (58 %) followed by triploidy (23%) and monosomy X (12%). An unbalanced structural rearrangement was found in one (2,3%) case. In two cases complex aneuploidies were identified: monosomy X simultaneously with trisomy 22, monosomy X with partial trisomy 6q. Most frequently involved in trisomies were chromosomes 16 and 22 (16% each), 4, 5, 15 and 21 (4, 7 % each). Trisomies of 2,10, and 13 chromosomes was found in one (2,3 %) cases each.

**Conclusions:** We concluded that FISH analysis, followed by CGH is a suitable technique for the detection of chromosomal abnormalities in spontaneous abortion. Future the results of molecular cytogenetic analysis of noncultured cells will provide additional insight and may even change our knowledge on the frequencies of chromosomal abnormalities in spontaneous abortions.

#### E-P01.21

#### Genetic and epigenetic factors of decreased ovarian reserve in patients from Ukraine

G. Livshyts<sup>1,2</sup>, O. Kotenko<sup>3</sup>, N. Hryshchenko<sup>1</sup>, K. Khazhylenko<sup>2</sup>, L. Livshyts<sup>1</sup>

<sup>1</sup>*Institute of Molecular Biology and Genetics NAS Of Ukraine, Kyiv, Ukraine, <sup>2</sup>ISIDA-IVF clinic, Kyiv, Ukraine, <sup>3</sup>Taras Shevchenko National University of Ukraine, Kyiv, Ukraine.*

Today more and more women delay the childbearing. Such a social shift results in increased number of women who get interested in having regular cycles and giving a birth at a later age but who are subfertile due to their diminished ovarian reserve. A search of genetic markers and X-chromosome (XCI) patterns are in great demand as it might help on the one hand, in identifying of women at a ovarian failure risk and, on the other, women who can benefit from advanced fertility preservation technologies applied prior to oocyte loss. The aim of our work was to study the association between phenotype and INH $\alpha$ , FMR1, FSHR, ESR1 genotypes and XCI-status in women with ovarian dysfunction and those with "poor response" to FSH ovarian stimulation in IVF cycles. It was shown that the frequencies of 769G>A in INH $\alpha$  variant and -397T Pvull variant in ESR1 gene heterozygous carriers, polymorphic haplotype Ala307-Ser680 heterozygous carriers, and "high

risk" alleles of FMR1 gene (40-47 CGG-repeats) heterozygous carriers were significantly higher in both ovarian dysfunction and "poor responders" groups comparing to control groups. According to analyses of XCI patterns in peripheral blood DNA samples of "poor responders" and control groups the frequency of skewed X-inactivation women are significantly higher in the "poor responders" group than in group of "good responders". Obtained results showed that the analyzed allelic variants in INH $\alpha$ , FMR1, FSHR, ESR1 genotypes and XCI-patterns can be used in genetic diagnostic of ovarian dysfunction and "poor" response to FSH stimulation in IVF-cycles.

#### E-P01.23

#### Vacuoles in sperm head are associated with DNA damage

E. Komarova<sup>1</sup>, M. Mazilina<sup>2</sup>, I. Fedorova<sup>1</sup>, A. Gogzyan<sup>1</sup>

*<sup>1</sup>The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O. Ott, Saint-Petersburg, Russian Federation, <sup>2</sup>St. Petersburg State University, Saint-Petersburg, Russian Federation.*

Sperm DNA quality plays a major role in male fertility. But the parameters of the conventional semen analysis do not reliably predict it. The integrity of the sperm DNA is essential for the accurate transmission of genetic information. Large sperm nuclear vacuoles are thought to be related to sperm DNA damage such as DNA fragmentation.

The purpose of this study was to know if the sperm morphology reflects its DNA fragmentation which thought to be associated with reduced embryo quality, implantation and live birth rates.

In the present study we evaluated the sperm DNA fragmentation measured by the deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL) in 10 male patients with subfertility. For each patient two types of motile spermatozoa were selected under the high magnification by the same trained operator as follows: with large nuclear vacuoles and normal. We included patients with various sperm characteristics (various proportions of 'normal' and 'vacuolated' spermatozoa in ejaculate, various motility profiles and various sperm concentration).

A total of 429 'vacuolated' and 197 'normal' spermatozoa were analysed. The threshold for statistical significance was set to  $P \leq 0.05$ . Among sperm with large nuclear vacuoles DNA fragmentation rate was extremely higher compare to the control: 12,6% vs. 1,5% ( $p < 0.001$ ).

The DNA fragmentation rate appeared to be correlated with the presence of large sperm-head vacuoles. Such a relationship might help to select spermatozoa with intact DNA to improve fertilization efficiency and embryo quality when assisted reproductive technologies are used.

#### E-P01.26

#### Analysis of maternal polymorphisms of MTHFR gene and risk of Down syndrome offspring

N. Keser, A. Pazarbaşı, L. Özpak, H. Lüleyap, H. Pazarbaşı, F. Özgünen

*Çukurova University Faculty of Medicine Department of medical Biology, Adana, Turkey.*

**BACKGROUND:** Down syndrome (DS) is the most common trisomy in live born with a prevalence of 1 in 1000 to 1 in 1100. Well-studied etiologies were maternal age and genetic polymorphism of folate metabolic pathway. Folate metabolism is governed by multiple enzymes. One of them methylenetetrahydrofolate reductase (MTHFR) converts 5,10-methylenetetrahydrofolate to 5 methylenetetrahydrofolate. Polymorphisms in gene encoding the folate metabolizing methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) have been linked to the etiology of Down syndrome. We examined the prevalence of these variant genotypes in mothers who had given birth to a child with Down syndrome and in control mothers.

**MATERIALS AND METHODS:** Two common variants C677T and A1298C of the MTHFR gene were screened in mothers with DS children ( $n = 67$  for C677T polymorphism;  $n = 65$  for A1298C polymorphism) and control mothers without DS children ( $n = 53$  for C677T polymorphism;  $n = 51$  for A1298C polymorphism) from Çukurova region of Turkey. The MTHFR genotypes were studied by RFLP analysis of PCR-amplified products.

**RESULTS:** AA and AC genotypes for A1298C polymorphism are not associated with the risk of DS pregnancy while C677T polymorphism is significantly associated with the risk of DS pregnancy. The CC genotype was at higher rate at cases than controls for A1298C polymorphism ( $p < 0.05$ ). The CC genotype was higher in the controls than cases for C677T polymorphism ( $p < 0.05$ ).

**CONCLUSION:** Our study suggests that C677T polymorphism is significantly associated with the risk of DS pregnancy. CC genotype for A1298C polymorphism is also associated with the risk of DS pregnancy.

**E-P01.30****Heterochromatin polymorphism does not appear to alter spermatogenesis in infertile men**

*A. Urbano<sup>1,2</sup>, I. Ochando<sup>1,2</sup>, M. Sánchez<sup>1,2</sup>, E. Montoya<sup>1,2</sup>, A. Segura<sup>4,2</sup>, J. J. López-Gálvez<sup>4,2</sup>, M. Lloret<sup>1,2</sup>, J. Rueda<sup>3,1,2</sup>,*

<sup>1</sup>Unidad de Genética. Clínica Vistahermosa, Alicante, Spain, <sup>2</sup>Cátedra de Biomedicina Reproductiva Vistahermosa. UMH, Alicante, Spain, <sup>3</sup>Departamento Histología. Universidad Miguel Hernández, Sant Joan d'Alacant, Spain, <sup>4</sup>Unidad de Reproducción. Clínica Vistahermosa, Alicante, Spain.

The number of infertile men is increasing in recent years. Heterochromatin polymorphism (HP) is a normal cytogenetic variant that is more frequent in infertile men. It has been suggested a relationship between HP and infertility, although not a possible mechanism. The HP could be related to the existence of meiotic alterations that can be detected by Fluorescent in situ hybridization (FISH) analysis in decondensed sperm. In the present work, we study blood karyotype and FISH on spermatozoa of infertile men, to evaluate the relation between heterochromatic variants and meiotic alterations. Blood karyotype and sperm aneuploidy (disomy and diploidy) rates for chromosomes 13, 18, 21, X and Y were evaluated in 251 infertile men with karyotype without structural rearrangement.

A total of 84 patients showed alterations in the FISH (33.5%) and 167 were normal. From the 251 patients, 52 patients (20.7%) showed HP. 22.6% of the patients with HP showed altered FISH in contrast to 19.7% of the patients with HP an normal sperm FISH. The most frequently observed polymorphism was 9qh+ (11%).

There were no significant differences in abnormal sperm FISH analysis between individuals with chromosomal polymorphisms and with normal karyotype (36.5% vs 32.7%; p>0.05).

Our data reveals that HP is not related to FISH alteration. Infertile men with HP have not more meiotic alteration than infertile men with normal karyotype, suggesting that alteration of spermatogenesis is not the origin of infertility in men with HP

**E-P01.31****The influence of gene-gene interactions and fibrinogen level on ART results in women with primary infertility**

*N. Medvedieva<sup>1</sup>, Z. Rossokha<sup>1</sup>, S. Kyryachenko<sup>1</sup>, I. Gyulmamedova<sup>2</sup>, V. Sirenko<sup>3</sup>, N. Gorovenko<sup>2</sup>,*

<sup>1</sup>State Institution "Reference-centre for molecular diagnostic of Public Health Ministry of Ukraine", Kyiv, Ukraine, <sup>2</sup>Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine, <sup>3</sup>The Institute for Reproductive Medicine, Kyiv, Ukraine.

Introduction: Unsuccessful ART procedure is associated with coagulation genes polymorphism. Fibrinogen is one of the most important factors in the coagulation cascade. Increased fibrinogen level can be influenced adversely on ART result.

Materials and methods: We analyzed past histories of 97 women with primary infertility after ART. The analysis included FV (G1691A), FII (G20210A), PAI-I (675 5G/4G), MTHFR (C677T, A1298C), MTRR (A66G), MTR1 (A2756G), FGB (C148T, G-455A), ESR1 (A-351G, T-397C) genes polymorphism and fibrinogen level investigations. MDR 2.0 and SPSS 17.0 programs were used for statistical analysis.

Results: 45 women had successful treatment of infertility during ART procedures (Group 1). No embryo implantation was in 52 women (Group 2). Fibrinogen levels were measured in women of both groups during 5-7 days after embryos transfer. The average level of fibrinogen in comparison groups did not differ. We have found two predictive model of ineffective ART procedure: MTHFR (677CT)/PAI-I (675 5G/4G)/FGB (C148T) and FGB / PAI-I(675 5G/4G)/fibrinogen level. Cut-off for unfavorable mean fibrinogen level was 3,9 g/l. Patients in Group 2 had significantly higher prevalence of 148 CT and 148 TT genotypes in FGB gene and fibrinogen levels more than defined cut-off. But we found no correlation between FGB polymorphism and fibrinogen.

Conclusions: The risk of ineffective ART procedure in women with primary infertility is significantly increased in case of combined influences of patients FGB (C148T) genotypes and higher fibrinogen level. Further investigations of gene-gene and gene-factors interactions are necessary to find group risk of unfavorable ART outcome.

**E-P01.32****Ascertainment of chromosomal imbalances origin: utility of parental chromosome and FISH analysis for genetic and reproductive counselling**

*E. Margarit<sup>1,2,3</sup>, M. A. Vallecillos<sup>1</sup>, A. Sánchez<sup>1,2,3</sup>, A. Soler<sup>1,2,3</sup>,*

<sup>1</sup>Biochemistry and Molecular Genetics and IDIBAPS, Hospital Clínic, Barcelona, Spain, <sup>2</sup>CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain, <sup>3</sup>IDIBAPS: Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain.

INTRODUCTION: The finding of a genomic imbalance provides an initial genetic diagnosis for a patient/fetus. The extension and composition of deleted/duplicated region determines his possible pathogenicity. The risk of associated abnormalities is higher when the alteration is de novo, an issue especially important in prenatal diagnosis. When the chromosomal imbalance has been originated by a parental balanced rearrangement, the recurrence risk for next pregnancies is significantly increased, particularly in complex alterations, insertions and some type of inversions.

METHODS: After reviewing our series of cytogenetic parental studies following chromosome imbalance detection, we report, among all the ascertained abnormalities, those particular cases demonstrating the importance of additional chromosome and FISH analyses in patients and parents to characterize and locate the unbalanced rearrangement.

RESULTS: In the following cases, additional investigations using conventional cytogenetic and FISH analysis resulted in a more accurate genetic diagnosis for affected individuals and risk assessment for future gestations, substantially increasing the quality of genetic counselling: ins(18;2)mat-t(8;13;12)- double t(14;15)(14';21)- rec inv(4)pat- rec inv(14)mat- rare segregation 3:1 for t(3;5)pat- del(Xp)mat- t(Y;autosome).

CONCLUSIONS: The finding of a genomic imbalance in a patient/fetus should be followed by additional studies to precise and locate the chromosomal rearrangement, in special when deletion or duplication has been identified using a quantitative analysis as array-CGH, qPCR or MLPA. Also, parental study is always required to investigate the aberration origin, very important not only for genetic prognosis but also for reproductive counselling.

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**E-P01.35****A genetic association study of AGT and AGTR1 gene polymorphisms in idiopathic spontaneous abortion in Iranian Patients**

*M. Sheikholeslami<sup>1</sup>, M. yavari<sup>1,2</sup>, M. Heidari<sup>1</sup>, M. Seyed Hasani<sup>2</sup>, M. Khatami<sup>1</sup>,*

<sup>1</sup>Department of Biology, Faculty of Science, Yazd University, yazd, Iran, Islamic Republic of, <sup>2</sup>Dr. Seyed Hasani medical Genetic Center, Yazd, Iran, Islamic Republic of.

Introduction: Recurrent spontaneous abortion is a multifactorial disease. At least 50 percent of abortions have no specific reason and are considered idiopathic. The renin-angiotensin system plays a considerable role in growth and differentiation in very early human development and in organogenesis. We sought to investigate whether exists any relationship between AGT M235T and AGTR1 A1166C with recurrent spontaneous abortion in Iranian patients.

Methods: the Frequency of AGT M235T and AGTR1 A1166C polymorphisms in 110 idiopathic recurrent spontaneous abortion women were compared to 105 females with no abortion history, employing tetra-primer ARMS-PCR. The validity of mentioned experiments were evaluated through sequencing procedure. The amino acid change was analyzed with tools like "UCL-CS", "EXPASY", "SIFT" and "Polyphen2".

Results: The frequency of A1166C in patients was AA (% 70), AC (% 28) and CC (% 1/8). The A1166C frequency of controls was AA (% 84/7), AC (% 15/2) and CC (% 0) (P=0.008) and the frequency of M235T in patients was TT (% 38.18), TC (% 61.8) and CC (% 0). The M235T frequency of control cases was TT (% 83.8), TC (% 16.2) and CC (% 0) (P=0.001).

Conclusion: Our results show significant relationship between the AGT M235T and the AGTR1 A1166C polymorphisms with idiopathic recurrent spontaneous abortion in Iranian women.

So these polymorphisms can serve as appropriate options for investigation of other populations and it may contribute to the understanding the one of the genetic etiology of idiopathic recurrent spontaneous abortion.

**E-P01.38****The possible role of chromosome 9q11.1-1.2 duplication resulting with infertility and recurrent pregnancy loss**

*O. Ozdemir, O. Yıldız, B. Kuru, B. Paksoy, D. Uysal, F. Silan;*  
*Faculty of Medicine, Canakkale, Turkey.*

Aim: Pericentric heterochromatic variation of chromosome 9 is frequently encountered in karyotyping analysis. However, 9q11.1-11.2 duplications ra-

rely reported in reproductive failure.

Method: 220 RPL and 145 infertility couples and 24 healthy reproductive controls (2 or more healthy children and none of miscarriage) were enrolled in the current results. Cytogenetic and molecular analyses using GTG-C banding and FISH were performed for the target chromosome 9q11.1-11.2 profiling.

First trimester fetal tissues of miscarriages analysed with QF-PCR and 13 aneuploid, 1 triploidy, 1 tetraploidy and 1 paternal-uniparental-isodisomy detected. Parents of abnormal fetuses analysed with GTG-C banding.

Results: 128 infertile/RPL patients (69 male-59 female) and only 1 healthy reproductive control's banded analysis and FISH results showed the homozygous (8/6.3%) or heterozygous (120/93.7%) 9q11.1-11.2 duplication and 1 case has 9q11.1-11.2 triplication with concordance in the current patient-control cohort with reproduction problems. 46, XY, 9qh11.1-11.2 triplication case's wife has two pregnancy loss, first with trisomy 15 and second with monosomy X; third pregnancy resulted with healthy girl.

9q11.1-11.2 duplication related to high risk of infertility/RPL (OddsRatio=4.89, p=0.1219)

All trisomy21(6 fetus), trisomy18(2), monosomyX and monosomy X+trisomy 21; triploidy(1), paternal-uniparental-isodisomy(1) fetuses has one or two 9qh+ parents; only trisomy 13(2) and tetraploidy(1) fetuses has parents without 9qh duplication. 9q11.1-11.2 duplication related to high risk of abnormal QF-PCR resulted fetuses (OddsRatio=23.00, p=0.0038)

Conclusion: Current results are the first report of 9qh+ variation in cases with RPL/infertility quantitative analysed with FISH and statistics compared to healthy reproductive cases. Results show the dup9q11.1-11.2 may cause the chromosomal abnormal fetuses because of interchromosomal effect and resulted as reproduction failure.

#### E-P01.40

##### Structural chromosome aberrations in couples with fertility problems

A. Kybartiene, Z. Zemeckiene, J. Peculiaitiene, D. Medelinskienė, V. Asmoniene;  
Department of Genetics and Molecular Medicine, The Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania.

Introduction. Infertility is important health problem, there are about 10-15% of infertile couples in the world. Structural chromosome aberrations may be one of the infertility reasons. The aim of this study was to evaluate structural chromosome aberrations in couples with fertility problems in Lithuania.

Materials and Methods. 187 couples with fertility problems (187 male (age 34,6±6,38 yrs) and 187 female (average age 32,0±5,51 yrs)), who underwent genetic counseling in The Hospital of Lithuanian University of Health Sciences Kauno Klinikos in 2013-2015y, were involved in this study.

Karyotype analysis from peripheral blood lymphocytes was performed for all couples by the standard laboratory protocol. Chromosome preparations were stained with trypsin-Giemsa to obtain G-banding. C-banding was also performed when necessary.

Results. Normal karyotype was found in 165 (88,2%) and chromosomes with structural aberrations in 22 (11,8%) of all tested couples. 14 (63,64%) of chromosome aberrations in couples were heteromorphic structural changes. We found 3 (1,60%) couples with chromosome alterations in both partners. Complete results are presented in table.

Conclusions. In this study we found that structural chromosome aberrations could influence infertility in only 11% of couples with fertility problems in Lithuania.

Chromosomal findings	Chromosome findings in tested couples with fertility problems	
	Women (n=187)	Men (n=187)
Normal chromosomes	n=174 (93,05%)	n=175 (93,6%)
Chromosomes with heteromorphic variants	n=8 (4,28%) 46,XX,1qh+ 46,XX,1qh+ 46,XX,inv(9)(p11q13) 46,XX,inv(9)(p11q13) 46,XX,15pss 46,XX,16qh+ 46,XX,16qh+ 46,XX,16qh+	n=9 (4,81%) 46,XY,9qh- 46,XY,9qh+ 46,XY,inv(9)(p11q13) 46,XY,14pss 46,XY,16qh- 46,XY,16qh+ 46,XY,21pss 46,XY,22pss 46,XY,22pss+
Sex chromosome abnormalities	n=2 (1,07%) mos45,X[4]/46,XX[96] mos45,X[3]/46,XX[51]	n=0 (0%)
Autosomal abnormalities	n=3 (1,60%) 46,XX,(2;5)(p22;q32?) 45,XX,der(13;14)(q10;q10) mos47,XX,+mar[5]/46,XX[65]	n=3 (1,60%) 46,XY,der(15)add(15)(p13?) mos47,XY,+21[4]/46,XY[46] mos47,XY,16qh+,+mar[5]/46,XY,16qh+[45]

#### E-P01.41

##### Double and triple hetero and homozygous forms of FVL/PTH/MTHFR mutations as a high risk factor of pregnancy complications in Georgian population

N. Pirtskhelani<sup>1</sup>, N. Kochiashvili<sup>1</sup>, K. Kartvelishvili<sup>1</sup>, L. Makhalidiani<sup>2</sup>, N. Pargalava<sup>3</sup>,

<sup>1</sup>National Forensics Bureau/Forensic Biology (DNA) Department, Tbilisi, Georgia,

<sup>2</sup>Hemophilia and Thrombosis Center, K. Eristavi Experimental and Clinical Surgery National Center, Tbilisi, Georgia, <sup>3</sup>Georgian Centre of Angiology and Vascular Surgery, Tbilisi, Georgia.

Introduction: Inherited thrombophilia, caused by the gene mutations of Factor V Leiden (FVL), Prothrombin (PTH G20210A) and Methylenetetrahydrofolate reductase (MTHFR C677T), is often associated not only with thromboembolism, but also with pregnancy complications, but we don't have detailed information about correlations between double and triple hetero and homozygous forms of FVL/PTH/MTHFR mutations and pregnancy complications (Spontaneous abortions, stillbirths, premature placental abruption, fetal development delay, preeclampsia, thromboembolism). Purpose of the study was to detect presence of different combinations of above mentioned mutations as a high risk factor for pregnancy complications.

Materials and Methods: 350 Patients with pregnancy complications and 100 healthy individuals (aged 20-45 years), were genotyped by PCR analyses.

Results: Different combinations of double and triple hetero and homozygous forms of FVL/PTH/MTHFR mutations (50 cases) were found in patients (27 (7,71%) cases of FVL/MTHFR heterozygous, 9 (2,57%) - PTH/MTHFR heterozygous, 6 (1,71%) - FVL-hetero/MTHFR-homozygous, 3 (0,86%) - FVL/PTH/MTHFR heterozygous, 2 (0,57%) - PTH-hetero/MTHFR-homozygous, 2 (0,57%) - FVL/PTH heterozygous, 1 (0,29%) - FVL homozygous mutations) and only one case with double PTH/MTHFR mutations in control group. There was a strong correlation between combinations of these mutations and development of thromboembolism, together with other complications, during pregnancy and postpartum.

Conclusions: Existence of these double and triple mutations indicates high distribution of inherited thrombophilia in Georgian population. Therefore we confirm a significant role of inherited thrombophilia, especially combined forms of mutations in development of pregnancy complications with severe thromboembolism during pregnancy and postpartum.

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#### E-P01.42

##### Lethal Gaucher Disease: a resolution for a challenging family history of intrauterine fetal deaths

N. Ekhilevitch<sup>1,2</sup>, A. Kurolap<sup>1</sup>, A. Mory<sup>1</sup>, T. Hershkovitz<sup>1</sup>, H. N. Baris-Feldman<sup>1,2</sup>,

<sup>1</sup>Rambam health care campus, Haifa, Israel, <sup>2</sup>Rappaport School of Medicine, Technion – Israel Institute of Technology, Haifa, Israel.

Introduction. We describe a young couple with a history of four in-utero fetal deaths at 7-23 weeks of gestation. All fetuses presented with severe IUGR, multiple congenital anomalies and fetal hydrops. The parents are first-degree cousins of Muslim-Arab origin from Northern Israel. They have two healthy daughters. Recurrent in-utero fetal deaths with severe fetal malformations have a wide differential diagnosis. Family history and consanguinity directed toward autosomal recessive inheritance; therefore, whole-exome sequencing was utilized.

Methods. Following parental informed consent, whole-exome sequencing was conducted on a fetus and both parents' genomic DNA using Illumina's NextSeq platform. Variant analysis focused on rare pathogenic homozygous changes, followed by Sanger validation and co-segregation analysis.

Results. Exome analysis revealed a homozygous missense mutation in the GBA gene, NM\_000157.3: c.820G>A (p.Glu274Lys).

Discussion. Gaucher disease is an autosomal recessive disorder with genetic and phenotypic heterogeneity caused by deficiency of glucocerebrosidase. The lethal type is typically depicted in-utero or during the neonatal period as hydrops and/or congenital ichthyosis with severe progressive neurological symptoms. The p.Glu274Lys mutation has previously been described as disease-causing in conjunction with another deleterious variant, showing a 100% decrease of GBA enzymatic activity; our family describes the first occurrence of this variant in a homozygous manner.

Lethal Gaucher disease is rarely diagnosed due to the clinical difficulty of recognizing it in-utero. Our family emphasizes that lysosomal storage diseases should be suspected in recurrent in-utero fetal deaths with severe fetal malformations and hydrops fetalis.

**E-P01.43****Inversion 4 Carrier with Infertility**

M. S. Yıldırım, M. N. Somuncu, A. G. Zamani;

Department of Medical Genetics, Meram Medical Faculty, Necmettin Erbakan University, KONYA, Turkey.

Inversion is one of the rearrangements result when a chromosome segment excises and reintegrates oriented 180° from the original orientation. There are two types of this chromosomal anomalies according to centromere position thus if the inversion segment includes centromere is called pericentric so break points are in each arm or thus if doesn't include centromere so both breaks occur in one arm is called paracentric inversion types. This chromosomal aberration usually may not cause any phenotypically effects on carriers as if the inversion segment is balanced but when the rearrangement is unbalanced with extra or missing DNA may due to miscarriage and infertility.

In our case, a 25 years old female represented by pericentric inversion on chromosome 4 applied of prediagnostic infertility also our case had been ivf pregnancy but resulted abortion. The patient peripheral blood was analyzed by classic cytogenetic technique as added to FISH method for detecting the orientation of the inverted segment. FISH probe was amount of the 4p(16.3) and 4q(ter) region but we didn't observed any inversion signal with fluorescently microscopy. At the end of cytogenetic analysis the karyotype was detected 46,XX,inv(4)(p16.2;q1,12).

Inversions are no loss of the genetic material however the gene sequences can be changed thus if crossing over occurs including inverted segments can cause the unbalanced gamets. In this case the patient karyotype can lead to an increased risk of miscarriage and infertility. So that family may be offered genetic counseling and comprehensive genetic examination.

**E-P01.45****Prenatal diagnosis and outcomes of congenital lower urinary tract obstruction (LUTO) in a South African hospital**H. Bezuidenhout<sup>1</sup>, L. Geerts<sup>2</sup>, M. Urban<sup>3</sup>;<sup>1</sup>Division of Molecular Biology and Human Genetics, Stellenbosch University, Cape Town, South Africa, <sup>2</sup>Department of Obstetrics and Gynaecology, Stellenbosch University, Cape Town, South Africa, <sup>3</sup>Division of Molecular Biology and Human Genetics, University of Stellenbosch, Cape Town, South Africa.

Introduction: To determine the frequency, etiology, survival and morbidity of prenatal Lower Urinary Tract Obstruction (LUTO) in a low-resource country.

Methods: Retrospective record review of prenatal LUTO cases at Tygerberg Hospital, South Africa, between January 2003 and June 2014.

Results: 75 prenatal LUTO cases were detected in 12 years, with a frequency of 1.2 per 10,000 births calculated over 3 years. The median gestation at diagnosis was 22.4 weeks. Prenatally 39 (52%) were classified as 'Isolated', 16 (21%) as 'Isolated with marker' and 20 (27%) as 'Complex'. Gender difference observed with predominance of males (60/68) (88%), male:female ratio 7.5:1. Males had predominantly 'Isolated LUTO' (n=36, 60%), and females 'Complex LUTO' (n=5, 63%). Survival outcomes included: TOP 26 (35%), IUD 1 (1%), Stillbirths 8 (11%), NND 12 (16%), Infant deaths 4 (5%), alive >1 year 16 (21%), Lost to follow-up/Unknown 8 (11%). The most common etiology was PUV (51%). Chromosomal aneuploidy was found in 9.3% (7/75), all in males, with Trisomy 21 the most common anomaly (4/7) (57%). Prenatal findings shown to be significantly associated with a 'Poor outcome' are bilateral renal cortex echogenic/cystic changes (p=0.029), anhydramnios (p=0.011) and pulmonary hypoplasia (p=0.003). Morbidity measures showed survivors beyond 1 year of age (n=16) had renal impairment (n=6, 37%), bladder dysfunction (n=4, 25%), recurrent UTI's (n=9, 56%).

Conclusions: Novel data on the burden of congenital LUTO in a developing country confirms high mortality and significant morbidity and supports the predictive value of specific ultrasound findings. The overall poor prognosis makes extensive counselling essential.

**E-P01.48****Rare case of male infertility due to monosomy X and structural rearrangement of chromosome Y**D. Brazdžiūnaitė<sup>1</sup>, B. Burnyte<sup>2</sup>, V. Sliuzas<sup>2</sup>, A. Utkus<sup>2</sup>;<sup>1</sup>Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Objective: To present a male with primary infertility due to mosaic karyotype with monosomy X and structural rearrangement of chromosome Y.

Inspection and Investigation: Here we present data from 34-year-old patient complaining about his inability to have children with his partner. There

were no significant health problems in childhood. Urological examination revealed testicular hypoplasia and his spermogram showed azoospermia. Phenotypically patient has short stature and eunuchoid voice. Screening of the AZFa, AZFb, and AZFc regions did not detect any chromosome Y microdeletions. Cytogenetic analysis of 50 metaphase spreads from peripheral blood lymphocyte cultures revealed mosaic karyotype: 45,X[44]/46,X,idic(Y) (p11.3)[16]. One cell line had monosomy X, while other had isodicentric chromosome Y, which was composed of two chromosomes Y fused at terminal segments of p arms. According to literature, this karyotype determines various male fertility disorders. Isodicentric chromosomes are the most commonly reported aberrations of the chromosome Y. Phenotypes of mosaic karyotype may vary from male to abnormal female or individual with ambiguous genitalia.

Conclusion: Although our patient has mosaic karyotype without normal karyotype cell line, he lives a normal life without physical symptoms or other complaints about health, except for primary infertility. It is well known that phenotypes depend on the location of chromosome breakpoints as well as on the proportion of each cell line and degree of mosaicism in various tissues.

**E-P01.53****MiRNA regulation of uterine fibroids pathogenesis.**

M. S. Matevosyan, I. O. Pokudina, D. E. Romanov, E. V. Butenko; Southern Federal University, Rostov-on-Don, Russian Federation.

Introduction. Uterine fibroids are the one of the most common neoplasms in women of reproductive age. The accumulated data and functional model experiments confirm the concept of the impact of microRNAs on disease development. MiRNAs regulate genes by binding to their transcripts, while the coefficient of miRNA binding is directly proportional to its regulatory capacity. Current study is to predict miRNA that regulate genes included in pathobiology of uterine fibroids.

Materials and methods. We analyzed the intergene spaces located in surroundings of 35 genes of the pathogenesis. Full sequences were obtained from NCBI data base using E-utilities API. miRNA sequences were taken from the miRBase release 21. Binding sites search was carried out with MiRanda and GLAM2Scan program combination. The results were filtered to yield only those matches with 95% identical nucleotides.

Results: The entire set of non-coding DNA sequences around 35 genes contains 164 miRNA binding sites of miR-619, miR-5096, miR-1273(g,h,e), miR-8485, miR-1273e, miR-5585, miR-548d -2, miR-1285-1 and miR-545. The maximum number of binding sites (13-20) was identified for HMG A2 (16), ICAM1 (20), TGFBI (17), MMP16 (20), KIT (13). MiR-619 regulates 22 genes from the study group, miR-5096 -18 genes, mir-1273g -13 genes, the rest miRNAs to regulate 1-3 genes studied.

Conclusion. The results suggest an important role that detected miRNAs play in uterine fibroids pathogenesis. Further research may provide the basis for future use of miRNAs as biomarkers and therapeutic targets.

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**E-P01.54****Prenatal cytogenetic analysis on amniocytes fails to detect low grade mosaic trisomy 9**A. Zagorac<sup>1</sup>, K. Witzl<sup>2</sup>, A. Golub<sup>1</sup>, Z. Kanic<sup>3</sup>, N. Kokalj Vokac<sup>1,4</sup>;<sup>1</sup>Laboratory of Medical Genetics, University Medical Centre Maribor, Maribor, Slovenia,<sup>2</sup>Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia,<sup>3</sup>Division of Paediatrics, University Medical Centre Maribor, Maribor, Slovenia, <sup>4</sup>Medical Faculty Maribor, University Maribor, Maribor, Slovenia.

The aim of prenatal diagnosis is to correctly establish and interpret the karyotype of the fetus. Detection of low-grade mosaicism involves many factors, like the type and number of tissues analyzed, the number of cells studied and the sensitivity of the techniques applied.

We describe a case of cytogenetic analysis after amniocentesis in which a normal male karyotype was obtained. Postnatal molecular cytogenetic tests on peripheral blood revealed a low level trisomy 9 mosaicism.

Amniocentesis requested for advanced maternal age gave a normal male karyotype. Because of poor fetal growth and the pathological CTG emergency cesarean section at 32nd week of gestation was done. At the age of 10 month the propositus was presenting some abnormalities: hypotonia, delayed motor development, mild micrognathia, deeper palmar and plantar creases, left eye strabismus, bilateral cryptorchidism and bilateral inguinal hernia. Ultrasound examination revealed ASD secundum, dilatation of the left ventricle, and bilateral mild pyelectasis.

These findings led us to perform postnatal molecular cytogenetic testing. Array-CGH on peripheral blood indicated mosaic trisomy 9. FISH on cultured peripheral blood lymphocytes using 9q subtelomeric DNA probe in com-

bination with CEP 9 centromeric DNA probe confirmed presence of cell line with trisomy 9 in 2% of metaphases and in 7% of interphase cells. Postnatal genetic tests results and boy's phenotype suggested that this is a case of low grade trisomy 9 mosaicism. We have compared the clinical findings of our patients with those previously reported and some thoughts about pitfalls in prenatal diagnosis will be discussed.

#### E-P01.56

##### Prenatal diagnosis of Pallister-Killian syndrome

D. Raskova<sup>1</sup>, V. Frisova<sup>2</sup>, D. Smetanova<sup>3</sup>, B. Prosova<sup>3</sup>, J. Hodacova<sup>1</sup>, M. Trkova<sup>4</sup>, V. Bevcarová<sup>1</sup>, L. Dohnalova<sup>1</sup>, M. Koudova<sup>1</sup>, D. Stejskal<sup>1</sup>;

<sup>1</sup>Centre for Medical Genetics and Reproductive Medicine GENNET, Prague 7, Czech Republic, <sup>2</sup>Profem-a-Fetal Medicine Centre, Prague 2, Czech Republic, <sup>3</sup>Dept. of Radiology 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague 5, Czech Republic.

Pallister-Killian syndrome is a rare chromosomal disorder characterized by a tissue-limited mosaicism of tetrasomy of the short arm of chromosome 12, caused by extra metacentric chromosome - isochromosome i(12)(p10). Mosaicism is present only in fibroblasts, the karyotype of lymphocytes is normal. Clinical features include intellectual disability, seizures, streaks of hypo- or hyperpigmentation, prominent forehead, flat occiput, hypertelorism, short nose with anteverted nostrils, flat nasal bridge, short neck, polyhydramnion, rhizomelic micromelia and diaphragmatic hernia.

Chorionic villi sampling (CVS) was performed at 28-year old G2/P0 woman due to nuchal translucency (NT) 3.5 mm/1.99 MoM and normal first trimester biochemistry. Karyotype and microarray investigation of the fetus were normal, screening for deletion of exons 7 and 8 of the SMN1 gene and mutations of the FGFR3 gene were negative.

The ultrasound investigation in 20th week proved mild ventriculomegaly, hydronephrosis bil., short long bones (under 5.percentile), high NSF, wide insertion of the umbilical cord with herniation of the liver tip, subcutaneous edema of face, skull and neck, edema of the nasal bridge, wide flat nose, flat profile, high forehead, hypertelorism, convulsive movements. Echokardiographic investigation and magnetic resonance were in accordance with ultrasound findings.

Differential diagnosis considerations included Pallister-Killian syndrome and repeated targeted prenatal diagnosis by amniocentesis was offered. The diagnosis of Pallister-Killian syndrome was conformed by finding of 80% mosaicism of tetrasomy 12p by FISH investigation (isochromosome of the short arm of chromosome 12).

The pregnancy was terminated in accordance with the decision of the family.

#### E-P01.62

##### Investigation of relationship between Interleukine-2 promoter polymorphism and preeclampsia

G. Ay, Ö. Turgut, U. Luleyap, M. A. Erkoc, P. Pazarci, D. Alptekin, F. T. Özgünen; Cukurova University Faculty of Medicine, Adana, Turkey.

Introduction: Pregnancy, which results in a healthy gestation period and birth depends on well-functioning immune system of mother. Interleukine-2 (IL-2) is one of the most important cytokine which is produced by active T-lymphocytes and determines the cellular or humoral response along with macrophages during infection. Preeclampsia is a disease which generally occur during first pregnancy and its potential reasons are considered to be genetic and immunologic factors.

Materials and Methods: In this study, we aimed to investigate the relationship between preeclampsia and polymorphisms at -384 position in the promoter region of IL-2 gene. For this purpose, blood samples are taken from patients with stable preeclampsia and from healthy people as control. Potential polymorphisms related to the -384 promoter region of IL-2 gene are investigated by using Restriction Fragment Length Polymorphism technique.

Results: In 89 preeclamptic women involved in study, 38, 36 and 15 of them are found to have TT, GT and GG polymorphisms respectively. In 57 pregnant women (control), 28, 22 and 7 of them are found to have TT, GT and GG polymorphisms respectively.

Conclusions: Allele frequencies and genotype distributions at -384 promoter region of IL-2 gene is analyzed by Chi-square test and no statistically significant difference between patient and control group is found. Since factors such as abnormal immunological tolerance in pregnant women during pregnancy, HLA discrepancy between father and mother, and imprinting are important in preeclampsia, we consider that genetic based comprehensive researches involving all negative factors effective in placenta formation will be useful.

#### E-P01.63

##### Investigation of Methylenetetrahydrofolate reductase (MTHFR) gene A1298C Polymorphism and Distribution of Genotypes in Preeclampsia and Normal Pregnancies

L. Özpak, A. Pazarbaş, N. Keser, N. Ilgaz, F. Özgünen; Çukurova University, ADANA, Turkey.

Introduction: Methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in the development of preeclampsia in pregnant woman might be thought to play an active role. Therefore, we assessed whether MTHFR gene A1298C polymorphism increase the risk of preeclampsia.

Method: In this study, 98 pre-eclamptic and 78 normotensive pregnant women were genotyped for MTHFR A1298C polymorphism by RFLP (restriction fragment length polymorphism) analysis and the distribution of genotype and allele frequencies belonging to this polymorphism in pre-eclamptic patients and controls were also evaluated.

Results: Among controls, the genotypes of A/A, A/C, and C/C were observed in 42%, 43%, and 15%, respectively, whereas the A/A, A/C, and C/C genotypes were observed in 46%, 50%, and 4% of case patients, respectively for MTHFR A1298C polymorphism. The C/C genotype of the MTHFR gene were associated significantly with the risk of developing preeclampsia. Prevalence of the CC mutant genotype (%15) for the A1298C polymorphism was higher among PE women. There was a significant difference in terms of these genotypes between preeclamptic and normotensive pregnant women ( $p=0.044$ ). There was significant difference in term of frequency of A allele ( $p<0.05$ ) while there was not significant difference for frequency of C allele. Conclusion: There was differences in genotype frequencies between preeclamptic patients and controls, but there was difference only in frequency of A allele between preeclamptic patients and controls, for MTHFR A1298C polymorphism.

#### E-P01.64

##### Association between P-Selectin polymorphism and preeclampsia

S. Sezer<sup>1</sup>, O. Ates<sup>1</sup>, F. Demirturk<sup>2</sup>, N. Bozkurt<sup>1</sup>, E. Ensari<sup>1</sup>;

<sup>1</sup>Department of Medical Biology, Medical Faculty, Gaziosmanpasa University, Tokat, Turkey, <sup>2</sup>Department of Obstetrics and Gynecology, Medical Faculty, Gaziosmanpasa University, Tokat, Turkey.

Introduction: Preeclampsia is a pregnancy-specific disorder characterized by new-onset hypertension and proteinuria. It is reported that the affecting 2-10% of all pregnancies. The disease develop after 20 weeks of gestation and the exact etiology is unknown.

However, the occurrence of the disease may be associated with apoptosis, early vascular and endothelial cell dysfunction. In the proposed another mechanism, changes in the immune system of pregnant as a result of increased inflammatory response is lead to faulty placentation. P-selectin is a cell adhesion molecule and it is increases in many inflammatory conditions including preeclampsia. Therefore, it may be important to study the selectin polymorphisms in preeclampsia patients. In this study, our aim was to research the possible association between P selectin (Thr715Pro) polymorphism and preeclampsia.

Materials and methods: The study group consisted of 101 preeclampsia patients and 113 woman controls. P-Selectin polymorphism were analyzed by using Polymerase Chain Reaction (PCR) -Restriction Fragment Length Polymorphisms (RFLP) method.

Results: Statistical evaluation of the data results showed a significant association for genotypic and allelic frequency distribution between P-Selectin polymorphism and preeclampsia ( $p=0.0027$ ;  $p= 0.004$ , OR: 4.03, 95% CI: 1.40 -14.12).

Conclusions: Our study results suggest that P-Selectin polymorphism may be one of the many genetic factors for preeclampsia susceptibility.

This work was supported by Gaziosmanpasa University Scientific Research Projects Fund

Allelic and genotypic distributions of the studied polymorphism				
	Patient Group n:101	Control Group n:113	p	OR (CI %)
P-selectin (Thr715Pro) Genotypes			0.0027	
Thr/Thr	98 [97.2%]	97 [85.8%]		
Thr/Pro	2 [1.9%]	15 [13.3%]		
Pro/Pro	1 [0.9%]	1 [0.9%]		
Allele frequency	198 [98.1%]	209 [92.5%]	0.004	4.03 (1.40 -14.12)
Thr	4 [1.9%]	17 [7.5%]		
Pro				

**E-P01.65****The endothelial protein C receptor haplotypes are not associated with increased risk of recurrent pregnancy loss or repeated implantation failure**I. Joksic<sup>1</sup>, S. Zivanovic<sup>2</sup>, G. Joksic<sup>2</sup>;<sup>1</sup>Clinic of Gynecology and Obstetrics, Belgrade, Serbia, <sup>2</sup>Institute of Nuclear Sciences Vinča, Belgrade, Serbia.

The endothelial protein C receptor (EPCR) is a key component of the protein C anticoagulant pathway. Four common haplotypes of PROCR have been reported (H1, H2, H3, H4). The A6936G allele (H3) is associated with lower EPCR densities on trophoblasts, but data are lacking for its effect on the risk of pregnancy loss in humans. In PROCR knockout mice, fibrin deposition in trophoblast cells results in thrombosis at the maternal-embryonic interface and pregnancy loss. Also, low EPCR density on trophoblast cells may influence implantation of the blastocyst and thus lead to implantation failure. We examined the frequencies of EPCR haplotypes in women experiencing recurrent pregnancy loss (RPL) and repeated implantation failure (RIF) and fertile controls.

The study included 50 women with history of at least three repeated IVF failures, 86 women with history of at least three pregnancies loss and 30 fertile controls. We investigated frequencies of EPCR gene haplotypes using reverse PCR Vienna lab CVD StrippAssays.

Our results showed no statistically significant difference in frequencies of EPCR haplotypes in three investigated groups (chi 2,003 p=0,8948). The most prevalent genotype is h1h2 in all studies groups, while h3h3 genotype has lowest frequency in our study population.

Although animal studies strongly suggested a link between EPCR haplotype and successful pregnancy outcome, our study did not confirm these findings. Further investigations on larger number of pregnant women as well as their partners is needed in order to evaluate if carrying a homozygous child is associated with increased risk of pregnancy loss

**E-P01.66****Cytokine and folate cycle genes additive effect study in pregnancy loss**

E. Mashkina, K. Kovalenko, E. Butenko;

Research Institute of Biology, Southern Federal University, Rostov-on-Don, Russian Federation.

When there are several gene products which are involved in one function and process, minor but multiple changes have additive effect and cause the disturbance of whole metabolic pathway. Here we investigate the additive effect of pro-inflammatory cytokines and folate cycle enzymes allele variants in pregnancy loss. 151 women with early pregnancy loss (ERL) and 134 women with normally progressing pregnancies were studied. Polymorphisms -31C-T (rs1143627) IL-1 $\beta$ , -174G-C (rs1800795) IL-6, C677T (rs1801133) MTHFR, A66G (rs1801394) MTRR were detected by allele-specific PCR. We analyzed gene-gene interactions among 4 polymorphisms using the multifactor dimensionality reduction (MDR) method. Two high risk genotypes were detected. 6,4% of women with EPL had genotype -31CT IL-1 $\beta$  / -174GG IL-6 / 677CT MTHFR / 66GG MTRR, whereas in control group this genotype wasn't detected. Described allele variants combination is a high-risk factor for the first trimester pregnancy loss (OR = 17,3 p = 0,01). 9,9% of women with EPL had genotype -31CC IL-1 $\beta$  / -174GC IL-6 / CT677 MTHFR / 66AG MTRR that is 3,9 fold higher rate than in control group (OR = 4,3 p = 0,03). Shift in inflammatory reactions intensity, including that caused by cytokines gene variants, is a risk factor for pregnancy. Pro-inflammatory cytokines can act as a mediators between inflammation and hypercoagulation. Hyperhomocysteinemia can cause the development of numerous pathologies through pro-inflammatory cytokines expression up-regulation, oxidative stress induction, apoptosis activation and methylation processes dysregulation. This study was supported by the federal assignment № 6.98.2014/K from Russian Ministry of Science and Education.

**E-P01.69****Small Supernumerary Marker Chromosome 15 identified in prenatal diagnosis due to advanced maternal age**S. Teofilova<sup>1</sup>, O. Miljanovic<sup>1</sup>, T. Ostojic<sup>1</sup>, M. Bulatovic<sup>1</sup>, T. Liehr<sup>2</sup>;<sup>1</sup>Clinical Center of Montenegro, Podgorica, Montenegro, <sup>2</sup>Institute of Human Genetics, Jena, Germany, Jena, Germany.

Introduction: Small supernumerary marker chromosomes are defined as extra and abnormal chromosomes whose derivation and content can be typically determined by G banding techniques. Approximately 70% of SMCs are de novo and 30% are inherited.

Materials and Method: Here, we report a case of sSMC identified in prenatal diagnosis. A 36-year-old pregnant woman was monitored due to advanced

maternal age. The amniocentesis was performed at 17 weeks of gestation. The fetal karyotype was obtained from an amniotic fluid cells.

Results: Conventional cytogenetics analysis revealed an abnormal karyotype 47,XY,+mar detected in all metaphases. Both parents had normal karyotypes. Molecular investigation by FISH were carried out in the Jena, Institute for Human Genetics. The additional marker chromosome could be characterized after the application of the probes mentioned above as a del (15) (q11.2). Molecular-cytogenetic result 47,XY,15p++mat,+ del (15) (q11.2) (100%). After a complete genetics analysis, the parents were informed that there are several cases small SMC(15)s described in the literature which did not show any clinical abnormalities regardless inherited or de novo, and those cases were not directly associated with an abnormal phenotype. After this information and ultrasound findings, the parents decided to continue with the pregnancy and keep the baby. In week 40 of the gestation a boy was born, now 3 years old, phenotypically healthy and with normal intelligence level.

Conclusions: In such cases it is necessary to do molecular investigation by FISH in order to exclude the presence of critical region of Prader Willi and Angelman syndromes.

**E-P01.70****Screening of high-risk pregnancies for chromosomal aneuploidies**D. Medelinškienė<sup>1</sup>, Z. Zemeckienė<sup>1</sup>, A. Kybartienė<sup>1</sup>, G. Stremaitienė<sup>2</sup>, V. Asmonienė<sup>2</sup>;<sup>1</sup>Department of Genetics and Molecular Medicine, The Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania, <sup>2</sup>Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Introduction. The aim of this study was to evaluate numerical and structural chromosome aberrations in amniotic fluid cells of different maternal age groups.

Materials and methods. This retrospective study involved 1163 pregnant women (age 16-51 years, average 35.5±5.63 yrs), who underwent prenatal genetic counseling in The Hospital of Lithuanian University of Health Sciences Kauno Klinikos in 2012-2015. All women had high risk for aneuploidy according to double, triple biochemical tests or ultrasound examination.

Amniotic fluid for prenatal analysis was obtained by transabdominal amniocentesis between 16-22 weeks gestation. Interphase fluorescence *in situ* hybridization for aneuploidy screening was performed on non-cultivated cells with Kreatech Poseidon probes according to manufacturers protocol. Preparation of chromosome slides G-banding using trypsin-Giemsa staining were performed according to standard laboratory methods.

Results. We found aneuploidies in 89 cases (7,65%) and heteromorphic structural aberrations in 18 (1,55%). Results in different age groups are presented in table.

Conclusions. Most of chromosome aneuploidies and structural aberrations were found in women over 35 years. The most common aneuploidy was 21 chromosome trisomy.

Table. Chromosome aneuploidies and structural aberrations in different maternal age groups.

Maternal age groups	Chromosome aneuploidies and structural changes, n(percentages from all abnormalities)					Total
	13 chromosome trisomy	18 chromosome trisomy	21 chromosome trisomy	Sex chromosome aneuploidies	Triplets	
≤ 29 years (n=195)	2 (2.25%)	4 (4.49%)	3 (3.37%)	5 (5.62%)	3 (3.37%)	22 (24.72%)
30 - 34 years (n=180)	1 (1.12%)	5 (5.62%)	4 (4.49%)	1 (1.12%)	0 (0%)	11 (12.36%)
35 - 39 years (n=481)	1 (1.12%)	2 (2.25%)	20 (22.47%)	0 (0%)	1 (1.12%)	28 (31.46%)
≥ 40 years (n=290)	3 (3.37%)	3 (3.37%)	13 (14.61%)	2 (2.25%)	0 (0%)	7 (7.87%)
Total	7 (7.87%)	14 (15.73%)	40 (44.94%)	8 (8.99%)	4 (4.49%)	89 (100%)

**E-P01.71****Cytogenetics study of 468 sample of products of conception**I. Tkach<sup>1</sup>, N. Huleyuk<sup>1</sup>, D. Zastavna<sup>1</sup>, A. Weise<sup>2</sup>, N. Kosyakova<sup>2</sup>, T. Liehr<sup>2</sup>, E. Ciszkowicz<sup>2</sup>;<sup>1</sup>Institute of Hereditary Pathology, NAMS of Ukraine, Lviv, Ukraine, <sup>2</sup>Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany,<sup>2</sup>Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland.

Introduction: The karyotype of a spontaneously aborted conceptus provides valuable clinical information. Chromosomal abnormalities (mostly aneuploidy) account for ~50% of fetal losses in the first 8-15 weeks of gestation. For later (15-24 weeks) losses the frequency is approximately 20 %. Most of

them are numerical aberrations such as polyploidy, trisomy, or monosomy X. Materials and Methods: Cytogenetics study of 468 samples products of conception was performed. G-banding cytogenetic results were obtainable in 153 cases. For the analysis of products of conception where banding analysis was not possible due to absence of metaphases mFISH analysis was performed. Interphase mFISH with the probe panel for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y was performed on 315 uncultured cell suspensions from spontaneous abortions samples.

Results: G-banding and molecular-cytogenetic karyotyping of products of conception identified numerical chromosomal abnormalities in 34.4% of cases with prevalence of autosomal trisomy – 15.0%, polyploidy – 9.8% and monosomy X – 8.1%, especially in pure form (7.6%). Among the autosomal trisomy abnormalities of chromosomes 16 (5.3%), 21 (2.8%) and 18 (2.4%) were the most prevalent.

Karyotype	G-banding results	mFISH results	Total results	
	n	n	n	%
Normal XY	67	87	154	32.9
Normal XX	36	117	153	32.7
monosomy X	-	35	35	7.6
47,XXX	1	1	2	0.4
trisomy X	1	1	2	0.4
trisomy 3	1	-	1	0.2
trisomy 13	-	1	1	0.2
trisomy 14	-	2	2	0.4
trisomy 15	1	6	7	1.5
trisomy 16	7	18	25	5.3
trisomy 18	5	6	11	2.4
trisomy 20	2	-	2	0.4
trisomy 21	6	7	13	2.8
trisomy 22	3	5	8	1.7
triploidy	23	19	42	9.0
tetraploidy	-	3	3	0.7
monosomy X[40]/ trisomy X[60]	-	1	1	0.2
monosomy X[96]/ disomy X[4]	-	1	1	0.2
monosomy X[77]/ disomy X[23]	-	1	1	0.2
monosomy 15[61]/ disomy 15[39]	-	1	1	0.2
disomy 21[38]/ trisomy 21[62]	-	1	1	0.2
monosomy 22[26]/ disomy 22[23]/ trisomy 22[51]	-	1	1	0.2
2n[63]/4n[37]	-	1	1	0.2
<b>Total</b>	<b>153</b>	<b>315</b>	<b>468</b>	<b>100</b>

Conclusion: Consequently, detected of chromosome aneuploidies in samples from products of conception is a key part of the investigations of reproductive failure in humans.

#### E-P01.73

#### Parental microRNA polymorphism and recurrent pregnancy loss: an association study in Iranian population

*m. amin-beidokhti<sup>1</sup>, r. mirfakhraie<sup>1</sup>, s. zare-karizi<sup>2</sup>, f. karamoddin<sup>3</sup>;*

<sup>1</sup>*Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of,*

<sup>2</sup>*Islamic Azad University Varamin Pishva Branch, varamin, Iran, Islamic Republic of,*

<sup>3</sup>*Shafa Hospital, semnan, Iran, Islamic Republic of.*

Introduction: Recurrent pregnancy loss (RPL) is defined as two or more pregnancy losses prior to 20 weeks from the last menstrual period. The medical evaluation of RPL is mainly focused on the maternal factors. However, paternally expressed genes may also play role in implantation and placenta quality. In addition, recent studies have revealed the role of miRNAs in RPL. The aim of the present study was to investigate the possible association between parental miR-499aT>C (rs3746444) polymorphism and RPL in Iranian couples.

Materials and methods: We conducted a case-control study of 366 Iranian people consisted of 166 couples with at least two unexplained consecutive pregnancy losses, 100 healthy men and 100 healthy women with at least one live birth and no history of pregnancy loss. Genotyping was performed using PCR-RFLP. Significant difference in distribution of miR-499a genotypes was found only in males from RPL cases in comparison to controls, with P-value of 0.0005 and odds ratio equal to 2.91 (95% CI: 1.58-5.33).

Conclusions: We provide evidence for association between genetic variation in paternal miR499aT>C polymorphism and recurrent pregnancy loss. Further studies will be required to validate the significance of the studied genetic variation in diverse ethnic populations.

#### E-P01.74

#### Frequency of sexually transmitted bacterial infections with *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Gardnerella vaginalis* in spontaneous abortions

*K. Slavova<sup>1,2</sup>, T. Stoycheva<sup>2</sup>, A. Alexeev<sup>1</sup>, A. Todorova<sup>1,2</sup>, V. Mitev<sup>4</sup>;*

<sup>1</sup>*Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria.*

Gonorrhoeae, Ureaplasma and Gardnerella are among the most common sexually transmitted bacterial infections which lead to spontaneous abortion. The aim of our research was to monitor the frequency of these infections among spontaneous abortions in Bulgarian population.

For this reason we examined endometrial tissue derived from 100 patients using PCR technique. Patients were divided into two groups - 1) control group of 40 patients with voluntarily interrupted pregnancy and 2) target group of 60 patients with spontaneous abortion for unspecified reasons. In one of spontaneous abortion cases (1.7%) we found *Ureaplasma parvum*. *Gardnerella vaginalis* was found in five of the patients with spontaneous abortion (8%) and eleven of the control group patients (27.5%). *Neisseria gonorrhoeae* and *Ureaplasma urealyticum* infections were not found. Current results demonstrate high frequency (10%) of several sexually transmitted bacterial infections in spontaneous abortion. More profound analysis of these results is needed by comparing with additional control group patients with successfully completed pregnancy, because of the high possibility of genital infections in this examined control group patients with voluntarily interrupted pregnancy.

The study of association between spontaneous abortions and sexually transmitted bacterial infections will help spontaneous abortion prevention using adequate treatment.

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#### E-P01.75

#### A novel mutation in *SPECC1L* found in a 28-year-old woman

#### diagnosed with Opitz G/BBB syndrome in adulthood and a history of two female fetuses with diaphragmatic hernia and anomalies of the internal genitalia

*I. Lund<sup>1</sup>, E. Bhoj<sup>2,3</sup>, P. Bogaard<sup>4</sup>, S. Leenskjold<sup>5</sup>, E. Zackai<sup>2</sup>, D. Li<sup>3</sup>, H. Hakonarson<sup>3</sup>, M. Petersen<sup>1</sup>, I. Nielsen<sup>1</sup>;*

<sup>1</sup>*Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark,*

<sup>2</sup>*Department of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, United States,*

<sup>3</sup>*Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, United States,*

<sup>4</sup>*Department of Pathology, Aalborg University Hospital, Aalborg, Denmark,*

<sup>5</sup>*Department of Gynecology and Obstetrics, Aalborg University Hospital, Aalborg, Denmark.*

Introduction: Opitz syndrome was first described in 1969 and is characterized by a variable expression of midline defects. In 2015 the causative gene *SPECC1L* (22q11.2) of the autosomal dominant form of the condition was described. We report a novel missense mutation in *SPECC1L* in a family with ocular hypertelorism, bicornuate uterus and diaphragmatic hernia.

Material and Methods: The proband, a 28-year-old woman, presented with prominent ocular hypertelorism at birth. Chromosome analysis revealed a normal 46,XX karyotype. She developed normally and was not genetically nor clinically diagnosed until she was referred to genetic counselling after having undergone termination of pregnancy due to fetal diaphragmatic hernia. Additionally, the fetus had a bicornuate uterus; a feature that was also reported in the proband herself. Before the diagnosis was established, the proband was pregnant again. Ultrasound examination and subsequent fetal autopsy revealed a phenotype similar to that of the first fetus. Chromosomal microarray and sequencing of *MID1* (X-linked Opitz syndrome) was performed as *SPECC1L* analysis was not offered in a clinical setting. Further diagnostics proceeded with hotspot Sanger sequencing of *SPECC1L* on a research basis.

Results: A novel de novo missense mutation in *SPECC1L*, c.1258G>A, was found in the proband and subsequently in the two fetuses.

Conclusion: This case demonstrates that a clinical diagnosis is paramount in the prenatal setting reducing turnaround time as a targeted analysis strategy can be applied. The bicornuate uterus and diaphragmatic hernia reported in this family broaden the spectrum of phenotypic features in Opitz and *SPECC1L* hypertelorism syndromes.

#### E-P01.76

#### The study of *STAG3* gene mutations and Y chromosome

#### microdeletions in Iranian patients with non-obstructive azoospermia

*S. Pouriamanesh, Z. Kamalian, M. Amin-Beidokhti, R. Mirfakhraie;*

*Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.*

Introduction: Azoospermia is defined as complete absence of sperm from ejaculate and approximately occurs in 10-15 percent of infertile men with abnormal semen analysis. Genetic factors including microdeletions in the Y chromosome and single gene mutations are contributed to non-obstructive azoospermia. Recently, genome-wide association studies have identified the *STAG3* gene as a strong candidate gene for human male infertility. The aim of this study was to investigate the incidence of AZF deletions and mutation analysis of the *Stag3* gene among Iranian infertile men with idiopathic non-obstructive azoospermia.

Materials and methods: A total of 122 Iranian azoospermic infertile men were selected. The presence of 11 sequence tagged site (STS) markers from

AZF region including sY81, sY84 and sY86 for AZFa; sY121, sY124, sY127 and sY134 for AZFb; and sY242, sY239, sY254 and sY255 for AZFc were investigated using multiplex polymerase chain reaction (M-PCR). Existence of possible mutations in exon 7 of Stag3 gene was also investigated using multitemperature single strand conformation polymorphism (MSSCP) method. One hundred fertile men were also studied as control group.

Results: Thirteen (10.66%) patients showed Y chromosome microdeletions and among these, deletion in AZFc region was the most frequent. However, no mutation was detected in the Stag domain coded by exon 7 of the STAG3 gene.

Conclusion: According to the results, in the studied population, the main causing factor in developing azoospermia was Y chromosome microdeletions. Therefore, we did not suggest STAG3 gene as a strong candidate gene in non-obstructive azoospermia.

#### E-P01.79

##### The case of der(5)t(5;14)mat caused by adjacent I meiotic segregation in prenatal diagnosis

**D. Yuksel<sup>1</sup>, O. Izci Ay<sup>1</sup>, E. M. Ay<sup>1</sup>, O. Tezo<sup>2</sup>, G. Dogru<sup>1</sup>, K. Cevik<sup>1</sup>, U. Karakas<sup>1</sup>, F. Cayan<sup>3</sup>, I. Buran<sup>1</sup>, E. M. Erdal<sup>1</sup>;**

<sup>1</sup>Mersin University, Faculty of Medicine, Department of Medical Biology, Mersin, Turkey, <sup>2</sup>Mersin University, Faculty of Medicine, Pediatrics Clinic, Mersin, Turkey, <sup>3</sup>Mersin University, Faculty of Medicine, Department of Gynecology and Obstetrics, Mersin, Turkey.

Introduction: Genetic material exchange between homologous chromosomes occurs in somatic and germ cells. This type of exchanges in certain cases may occur in non-allelic chromosomal regions resulting in structural chromosomal anomalies in form of translocations. Translocations are categorized in two general types namely balanced and unbalanced. Balanced reciprocal translocations can risk the pregnancy as it may produce unbalanced gametes.

Materials and Methods: The amniotic fluid (20ml) belonged to a 27 years old female patient in 18th week of her pregnancy who was admitted to the Polyclinic of Gynecology and Obstetrics at Faculty of Medicine in Mersin University was sent to laboratory for cytogenetic analysis. Parents were not consanguineous. Amniotic material was processed using the in-situ culture method and GTG banding technique. 39 metaphase plates (20 colonies) were obtained and cytogenetic analyses performed on these plates.

Results: As a result of the cytogenetic analysis, the fetus was determined to be 46,XX,der(5)t(5;14)(p13;p11)mat karyotype. The cytogenetic analysis that was performed on peripheral blood samples taken from the parents showed that the finding was not de novo but of maternal origin.

Conclusions: Incidence of reciprocal translocations in the amniocentesis is 0.06%. Balanced reciprocal translocations do not change the amount of chromosomes and genetic materials. However they may cause unbalanced chromosomal rearrangement in the gametes of these carriers. Although the reciprocal translocation in our case is unbalanced. Upon family's request, the pregnancy was sustained. The 28-month case with Cri du Chat symptoms are being monitored by the Department of Pediatrics, Mersin University Faculty of Medicine.

#### E-P02 Sensory disorders (eye, ear, pain)

#### E-P02.01

##### Novel mutation in the CHST6 gene causes macular corneal dystrophy in a black South African family

**N. Carstens<sup>1</sup>, S. Williams<sup>2</sup>, T. Carmichael<sup>2</sup>, M. Cheung<sup>3</sup>, S. Büchmann-Möller<sup>2</sup>, M. Sultan<sup>3</sup>, E. Staedtler<sup>3</sup>, C. Zou<sup>4</sup>, P. Swart<sup>5</sup>, D. S. Rice<sup>5</sup>, A. Lacoste<sup>5</sup>, K. Paes<sup>5</sup>, M. Ramsay<sup>1</sup>;**

<sup>1</sup>Sydney Brenner Institute for Molecular Bioscience, University of the Witwatersrand, Johannesburg, South Africa, <sup>2</sup>Division of Ophthalmology, Department of Neurosciences, University of the Witwatersrand, Johannesburg, South Africa, <sup>3</sup>Biomarker Development, Novartis Institutes for BioMedical Research, Basel, Switzerland, <sup>4</sup>Center for Proteomic Chemistry, Novartis Institutes for BioMedical Research, Basel, Switzerland, <sup>5</sup>Novartis Institutes for Biomedical Research, Cambridge, MA, United States.

Macular corneal dystrophy (MCD) is a rare autosomal recessive disorder that is characterized by progressive corneal opacity that starts in early childhood and ultimately progresses to blindness in early adulthood. The aim of this study was to identify the cause of type I MCD in a black South African Sotho-speaking family with two affected sisters using whole exome sequencing. Variant filtering to identify the MCD-causal mutation included the inheritance pattern, variant minor allele frequency and potential functional impact. Ophthalmologic evaluation of the cases revealed a typical MCD phenotype and none of the other family members were affected. Variant filtering identified a homozygous E71Q mutation in CHST6, a previously identified MCD-causing gene encoding corneal N-acetyl glucosamine-6-O-

sulfotransferase, as the MCD-causal mutation in this family. This E71Q mutation results in a non-conservative amino acid change in a highly conserved functional domain of the human CHST6 that is essential for enzyme activity. This is the first description of MCD in a black Sub-Saharan African family and therefore contributes valuable insights into the genetic aetiology of this disease, while improving genetic counselling for this and potentially other MCD families.

#### E-P02.02

##### A novel FOXC1 mutation in an Axenfeld-Rieger patient with childhood glaucoma

**S. Carmona<sup>1,2</sup>, M. Freitas<sup>3</sup>, H. Froufe<sup>1</sup>, M. Simões<sup>1</sup>, M. Sampaio<sup>4</sup>, E. Silva<sup>5</sup>, C. Egas<sup>1,6</sup>;**

<sup>1</sup>Next-Gen Sequencing Unit, UC-Biotech, Cantanhede, Portugal, <sup>2</sup>Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>3</sup>Department of Ophthalmology, Hospital da Arrábida, Porto, Portugal, <sup>4</sup>Department of Paediatric, Hospital CUF, Porto, Portugal, <sup>5</sup>Vision Psychophysiology Unit, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, <sup>6</sup>Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

Axenfeld-Rieger syndrome (ARS) is a rare dominant, autosomal developmental disorder characterized by anterior segment abnormalities of the eye. Posterior embryotoxon with iridocorneal adhesions are always present and iris stromal thinning or atrophy, corectopia, iris holes and iris ectropion are common. Approximately half of the ARS patients develop glaucoma. Systemic anomalies like craniofacial anomalies and cardiovascular can also be present. Mutations and CNV in FOXC1 and PITX2 have been previously reported in ARS patients.

A DNA sample of a 14-month-old male with bilateral glaucoma, posterior embryotoxon with extension of the peripheral iris to Schwalbe's line, short stature, hypertelorism, frontal prominence, broad flat nasal root, maxillary hypoplasia and unilateral testicular atrophy was sequenced for the whole exome with the Ion Proton sequencer. Rare variants with functional impact were selected to identify the disease-causing variant. Sanger sequencing was performed to validate the mutation in the patient and to study the parents.

A novel heterozygous nonsense variant Y64X (c.192 C>G) was found in the FOXC1 gene. This de novo mutation leads to the loss of 88.6% of the protein, including the functional FHD domain.

The presence of de novo FOXC1 mutation confirms the ARS diagnosis. This result is consonant with previous studies that report a positive association between FOXC1 mutations and ARS patients with glaucoma. Further studies will be needed to understand the pathogenic mechanism of this mutation.

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#### E-P02.03

##### Novel BMP4 mutation causing coloboma in a Jewish Ashkenazi kindred

**Y. Yoge<sup>1</sup>, O. Wormser<sup>1</sup>, D. Halperin<sup>1</sup>, Y. Perez<sup>1</sup>, M. Volordarsky<sup>1</sup>, L. Gradstein<sup>2</sup>, O. S. Birk<sup>1</sup>;**

<sup>1</sup>Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev (NIBN), Ben-Gurion University of the Negev, Be'er Sheva, Israel, <sup>2</sup>Department of Ophthalmology, Soroka University Medical Center, Be'er Sheva, Israel, <sup>3</sup>Institute of Genetics, Soroka University Medical Center, Be'er Sheva, Israel.

An Israeli family of Jewish Ashkenazi descent presented with a phenotype of bilateral coloboma affecting several generations in an apparent dominant mode of inheritance. Whole exome sequencing data of an affected individual were analyzed and filtered for known benign variants using our in-house databases along with open access databases (1000 genomes, NHLBI ESP, ExAC etc.). The analysis yielded several candidate variants in genes which were previously associated with various ocular disorders. Candidate variants were further analyzed using Sanger sequencing and restriction analysis. Only a single heterozygous c.392A>G missense mutation in BMP4, resulting in a p.H121R substitution, showed full segregation within the family and was not found in 100 healthy Ashkenazi controls. In-silico analysis of the novel p.H121R variant showed that it is likely to have a deleterious effect on the mature protein. Mutations in BMP4 were previously described as causative for various conditions such as anophthalmia-micropthalmia, coloboma, retinal dystrophy, myopia, cleft lip, cleft palate and brain-digital abnormalities. Our data suggest that the novel BMP4 heterozygous mutation is the cause for the dominantly inherited isolated coloboma in this kindred, possibly through a dominant negative effect, in line with the known function of BMP4 as a homodimer.

## E-P02.04

**Whole exome sequencing of the Yakut family from Eastern Siberia with congenital autosomal-recessive cataract: the novel nonsense mutation in the FYCO1 gene**

N. A. Barashkov<sup>1,2</sup>, F. A. Konovalov<sup>3,4</sup>, F. M. Teryutin<sup>1,2</sup>, A. V. Solovyev<sup>1,2</sup>, V. G. Pschennikova<sup>1,2</sup>, G. P. Romanov<sup>1,2</sup>, N. N. Gotovtsev<sup>1</sup>, I. V. Morozov<sup>5,6</sup>, A. A. Bondar<sup>5</sup>, L. U. Dzhemileva<sup>7,8</sup>, E. K. Khusnutdinova<sup>7,9</sup>, A. N. Alexeev<sup>10</sup>, M. I. Tomsky<sup>1</sup>, O. L. Posukh<sup>6,11</sup>, S. A. Fedorova<sup>2,11</sup>

<sup>1</sup>Yakut Scientific Centre of Complex Medical Problems, Siberian Branch of the Russian Academy of Medical Sciences, Yakutsk, Russian Federation, <sup>2</sup>M.K. Ammosov North-Eastern Federal University, Yakutsk, Russian Federation, <sup>3</sup>Federal State Budgetary Institution „Research Centre for Medical Genetics”, Moscow, Russian Federation, <sup>4</sup>Genomed Ltd, Moscow, Russian Federation, <sup>5</sup>Institute of Chemical Biology and Fundamental Medicine of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation, <sup>6</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>7</sup>Institute of Biochemistry and Genetics of Ufa Scientific Centre, Ufa, Russian Federation, <sup>8</sup>Bashkir State Medical University, Ufa, Russian Federation, <sup>9</sup>Bashkir State University, Ufa, Russian Federation, <sup>10</sup>Institute of Humanitarian Research and Indigenous Peoples of the North, Siberian Branch of the Russian Academy of Sciences, Yakutsk, Russian Federation, <sup>11</sup>Federal Research Center Institute of Cytology and Genetics, Novosibirsk, Russian Federation.

Congenital cataracts are a major cause of vision loss in children worldwide. Autosomal -recessive congenital cataracts form a clinically diverse and genetically heterogeneous group of the crystalline lens - disorders. To identify the genetic cause of congenital nuclear cataract in Yakut family with three affected siblings from isolate Yakut population in Eastern Siberia (Sakha Republic, Russia) we performed whole exome sequencing on Illumina NextSeq 500 in one proband. For the first time, we have revealed homozygous c.1621C>T (chr3:46009205G>A) transition in previously known in association with congenital cataract gene FYCO1 (3p21.31). Detected mutation is not reported in the „1000 Genomes”, the ESP6500, and the ExAC projects. The c.1621C>T transition leads to premature stop-codon formation (p.Gln541Ter, NM\_024513.3) in exon 8 of FYCO1 gene. The c.1621C>T (p.Gln541Ter) mutation was found in homozygous state in one patient with congenital cataract (CTRCT18, OMIM: 610019) from family with three affected siblings whose mother and father have a normal vision. This finding confirms autosomal recessive pattern of cataract inheritance in examined Yakut family. Hence, by analogy of founder effect in prevalence of rare orphaned diseases described in Yakut population isolate such as SCA1 (OMIM 164400), methemoglobinemia (OMIM 250800), DM1 (OMIM 160900), three M syndrome 3 (OMIM 273750), OPMD (OMIM 164300) and DFNB1A (OMIM 220290) we suggest that this mutation in homozygous state is one of the major causes of congenital cataract in patients from Eastern Siberia. Study was supported by Ministry of Education and Science of the Russian Federation #6.656.2014/K and Sakha Republic Government project “Yakutian history”.

## E-P02.05

**Whole exome sequencing detects a probable novel mutation in the cluster of crystallin genes in a Mexican family with autosomal dominant congenital cataract**

O. Messina-Baas<sup>1</sup>, L. Gonzalez-Huerta<sup>2</sup>, J. Toral-Lopez<sup>3</sup>, S. Cuevas-Covarrubias<sup>4</sup>, <sup>1</sup>Oftalmologia, Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Genetica, Hospital General de Mexico, Mexico DF, Mexico, <sup>3</sup>Ecatepec Medical Center, Edomex, Mexico, <sup>4</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.

Introduction: Autosomal dominant congenital cataract (ADCC) is a clinically and genetically heterogeneous disease with high penetrance. ADCC represents around 10% of a treatable cause of childhood blindness. More than 30 loci have been associated to ADCC. Gamma crystallin genes are a family of 6 genes (4 CRYBA and 2 CRYBB) located on several chromosomes, this family of genes is associated to congenital cataract. The aim of the present study is to describe a Mexican family affected with ADCC and clinical diversity in three generations through whole exome sequencing (WES). Material and methods: Genomic DNA was analyzed through WES and DNA sequencing. Results: we detected a dominant CRYGA gene defect in exon 2 that produced the p.N18I change. This transversion mutation (c.53A>T) only co-segregated in the affected members of the family and was not found in non-affected members and in 100 normal controls. Bioinformatic analysis detected this mutation as deleterious. This missense mutation has not been previously reported; nevertheless more studies are necessary to confirm this type of mutations as deleterious. This is the first Mexican family ADCC with this mutation. WES is a powerful tool for the detection of genetic origins of cataracts due to genetic and clinical heterogeneity in this type of disease. Grant DGA-PA/PAPIIT/UNAM Proyect IN204114-2.

## E-P02.07

**Identification of a digenic inheritance in three Mexican families with hearing impairment using whole exome sequencing**

M. Martinez-Saucedo<sup>1</sup>, M. Rivera-Vega<sup>1</sup>, L. Gonzalez-Huerta<sup>1</sup>, H. Urueta-Cuellar<sup>1</sup>, P. Berruecos-Vilalobos<sup>2</sup>, M. Arenas-Sordo<sup>3</sup>, S. Cuevas-Covarrubias<sup>4</sup>;

<sup>1</sup>Genetica, Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Audiologia y Foniatria, Hospital General de Mexico, Mexico DF, Mexico, <sup>3</sup>Genetica, Instituto Nacional de Rehabilitación, Mexico DF, Mexico, <sup>4</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.

Background: Sensorineural hearing loss (SNHL) is a clinically and genetically heterogeneous disease. In some populations, mutations in the GJB2 gene represents the most frequent cause of hereditary SNHL. The great diversity of mutations in the GJB2 gene worldwide highlights the participation of ethnic background in SNHL. It is considered that 200 to 250 genes are involved in SNHL. Recently, more than 80 genes, around 1000 mutations, and 140 loci have been associated with SNHL (<http://hereditaryhearingloss.org/>). Improvement of the technology plays an important role in the diagnosis of different pathologies, whole exome sequencing (WES) represents an important tool in the identification of the genetic etiology in individuals with SNHL. Objective: To describe the presence of digenic inheritance in three families with SNHL involving the GJB2, MYO1C, OTOA and OTOG genes in a Mexican family with SNHL. Materials and methods: Three Mexican families with SNHL were included in the study. Analysis through WES and DNA direct sequencing analysis in all members of the family and in 100 normal controls Results: Affected sibs showed the digenic inheritance involving OTOA (p.E787X), GJB2 (c.365delG), MYO1C p.E831K and OTOG (p.T1235P) genes. Parents and non-affected members of the families were heterozygous for the molecular defect, all of them with normal audition. Conclusion: We describe three families through WES analysis with SNHL due to digenic inheritance; this enriches the mutational spectrum in Mexican population in patients with SNHL. Grant DGAPA/PAPIIT/UNAM Proyect IN204114-2.

## E-P02.09

**Fuchs endothelial corneal dystrophy: strong association with rs613872 not paralleled by changes in corneal endothelial TCF4 mRNA level**

D. Ozieblo<sup>1</sup>, E. Ruszkowska<sup>1</sup>, M. Udzila<sup>2</sup>, E. Binczyk<sup>2</sup>, A. Sciezynska<sup>3</sup>, R. Ploski<sup>4</sup>, J. P. Szaflak<sup>2</sup>, M. Oldak<sup>1,3</sup>;

<sup>1</sup>Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland, <sup>2</sup>Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland,

<sup>3</sup>Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland, <sup>4</sup>Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland.

Fuchs endothelial corneal dystrophy (FECD) is a common corneal endotheliopathy with a complex and heterogeneous genetic background. Different variants in the TCF4 gene have been strongly associated with the development of FECD. TCF4 encodes the E2-2 transcription factor but the link between the strong susceptibility locus and disease mechanism remains elusive. Here, we confirm a strong positive association between TCF4 single nucleotide polymorphism rs613872 and FECD in Polish patients (OR = 12.95, 95% CI: 8.63-19.42,  $\chi^2 = 189.5$ ,  $p < 0.0001$ ). We show that TCF4 expression at the mRNA level in corneal endothelium ( $n = 63$ ) does not differ significantly between individuals with a particular TCF4 genotype. It is also not altered in FECD patients as compared to control samples. The data suggest that changes in the transcript level containing constitutive TCF4 exon encoding the amino-terminal part of the protein seem not to contribute to disease pathogenesis. However, considering the strong association of TCF4 allelic variants with FECD, genotyping of TCF4 risk alleles may be important in the clinical practice.

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## E-P02.12

**Genetic analysis of rare non-syndromic prelingual hearing disorders**

R. Birkenhäger, C. Heinemann, S. Arndt, A. Aschendorff, R. Laszig;

Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center Freiburg, Freiburg, Germany.

Introduction: Approximately 1-3 / 1000 newborns are affected at birth or in the first two years of a profound hearing impairment, 60% of these cases are due to genetic causes. So far, 103 loci and 59 genes have been identified for this type of hearing loss. Genetic alterations in DFNB1 locus in which the genes GJB2 (connexin 26) and GJB6 (connexin 30) are located, are the main

cause of prelingual non-syndromic hearing loss. Objective of the project is to analyze, which other genes are involved in prelingual hearing disorders, in addition to the genes GJB2 and GJB6.

**Material and Methods:** In this project, so far 155 patients were included; a serious non-syndromic hearing impairment was diagnosed until the age of 2 years, also there were demonstrably no inner ear malformations and no alterations in the DFNB1 locus. The detection of genetic changes was carried out by bi-directional sanger sequencing of the coding exons, and intron transitions.

**Results:** First, the genes GRXCR1 and TPRN were in this patient population analyzed, and additionally the genes ESRRB, CIB2 and ILDR1. By DNA sequencing 3 novel mutations, 8 unknown polymorphisms and 22 known variations were detected.

**Conclusions:** The genetic analysis illustrated a few functionally relevant mutations in the genes GRXCR1 and TPRN, and additionally some unknown polymorphisms, an accumulation of changes in the analyzed genes is not present. Therefore further studies are required for the characterization of the etiology of rare genetic hearing disorders.

#### E-P02.16

##### Genetics of ion homeostasis in Ménière's Disease

**L. Zagato<sup>1</sup>, S. Delli Carpin<sup>1</sup>, C. Lanzani<sup>1</sup>, N. Casamassima<sup>1</sup>, C. Cassandro<sup>2</sup>, R. Albera<sup>2</sup>, M. Bussi<sup>1</sup>, P. Manunta<sup>3,4</sup>, R. Teggi<sup>1</sup>;**

<sup>1</sup>San Raffaele Scientific Institute, Milano, Italy, <sup>2</sup>Surgical Sciences Department, University of Torino, Torino, Italy, <sup>3</sup>Università Vita-Salute San Raffaele, Milano, Italy.

**Background and aims:** Ménière's Disease (MD) is an inner ear disorder characterized by episodic vertigo, fluctuating sensorineural hearing loss and aural fullness. It may arise from the interplay of genetic and environmental factors. To date the genetic investigation didn't produce conclusive results. Our previous work suggested a role for alpha-Adducin, a cytoskeletal protein in regulating the  $\text{Na}^+ \text{-K}^+$  pump activity, in MD patients. Since ionic homeostasis in the inner ear is crucial for the maintenance of endocochlear potential, in this work we studied the variants of genes involved in the regulation of ionic transport.

**Materials and methods:** 155 patients with definite MD were enrolled and accurately phenotyped. 137 control subjects without a lifetime history of vertigo were included. 36 SNPs located in 25 genes involved in ionic transport have been selected from a panel of polymorphisms associated to essential hypertension. Correction for multiple comparison tests has been performed applying a false discovery rate of 10%. Logistic regression analysis has been implemented to compute odds ratio, genetic interactions and genetic score effect.

**Results:** Four SNPs in 3 genes (SLC8A1, CYP11B2 and SIK1) displayed a significant difference in allelic and genotypic frequency ( $p=0.01/0.006$ ;  $p=0.028/0.009$ ;  $p=0.009/0.016$  and  $p=0.024/0.01$ ) in MD patients compared to controls. The presence of all four risk variants increases by 5 times the odds of developing MD. CYP11B interacts with SIK1 ( $p=0.002$ , OR=1.87).

**Conclusions:** genetic alterations of ionic transporters may act as a predisposing factor to develop MD. Further studies on other cohorts may validate these new candidate genes for MD.

#### E-P02.17

##### Ophthalmological phenotype associated with homozygous null mutation in the NEUROD1 gene

**O. Orosz<sup>1</sup>, M. Czeglédi<sup>2</sup>, I. Kántor<sup>2</sup>, I. Balogh<sup>3</sup>, A. Vajas<sup>1</sup>, L. Takács<sup>1</sup>, A. Berta<sup>1</sup>, G. Losonczy<sup>1</sup>;**

<sup>1</sup>Department of Ophthalmology, University of Debrecen, Debrecen, Hungary,

<sup>2</sup>Department of Ophthalmology, Jósa András Hospital, Nyíregyháza, Hungary, <sup>3</sup>Division of Clinical Genetics, University of Debrecen, Debrecen, Hungary.

**Introduction:** NEUROD1 is a basic helix-loop-helix protein involved in the development and maintenance of the endocrine pancreas and neuronal elements. Loss of NEUROD1 causes ataxia, cerebellar hypoplasia, sensorineural deafness and retinal dystrophy in mice. Heterozygous loss-of-function mutations in NEUROD1 have previously been described as a cause of maturity-onset diabetes of the young (MODY). Homozygous NEUROD1 mutations have only been detected in three patients causing permanent neonatal diabetes, neurological defects, including visual impairment. Our aim was to characterize the ophthalmological phenotype associating to the previously reported homozygous c.427\_428delCT NEUROD1 mutation.

**Materials and Methods:** The female patient was investigated on multiple occasions for 6 years, including visual acuity testing, automated perimetry, funduscopy, anterior-segment imaging, optical-coherence tomography of the posterior pole, standard full-field electroretinography, and fundus-autofluorescence imaging.

**Results:** The patient suffered from nyctalopia, blurry vision, visual-field con-

striction from childhood. Her best corrected visual acuity ranged between 20/25 and 15/25. Perimetry showed concentric constriction in both eyes. Optical-coherence tomography revealed total absence of the photoreceptor layer of the retina outside the fovea, where a discoid remnant of cone photoreceptors could be detected. The standard full-field electroretinography could not detect electrical response from the retina. Color fundus photos presented peripheral chorioretinal atrophy and central RPE mottling. A hyperreflective parafoveal ring was detected on fundus autofluorescent photos.

**Conclusions:** Loss of NEUROD1 has similar functional and anatomical consequences in the human retina as are described in mice. The present description can help the diagnosis of future cases and provide clues on the rate of disease progression.

#### E-P02.18

##### Population frequencies of 35delG mutation in GJB2 gene in main ethnic groups of Karachay-Cherkess Republic

**N. E. Petrina<sup>1</sup>, A. K. Makaov<sup>2</sup>, N. V. Petrova<sup>1</sup>, T. A. Vasilyeva<sup>1</sup>, A. N. Petrin<sup>3,4</sup>, E. K. Ginter<sup>4</sup>, R. A. Zinchenko<sup>1,4</sup>;**

<sup>1</sup>Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>2</sup>Municipal Budgetary Health Care setting „Habetskaya central district hospital”, Chabez, Russian Federation, <sup>3</sup>Moscow State University of Medicine and Dentistry, Moscow, Russian Federation, <sup>4</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

**Introduction:** Hearing loss is one of the most common human diseases. The frequency of hearing loss is 1:1000-2000 newborns. About 50% of congenital deafness is caused by hereditary factors. More than 100 loci associated with hereditary nonsyndromic deafness (HND) have been mapped, among which the most has AR type of inheritance. More than half of all cases of early-onset HND in European populations is due to a 35delG mutation in the GJB2 gene. However, 35delG frequency varies in populations and ethnic groups.

**Materials and Methods:** 766 healthy individuals of the main ethnic groups of Karachay-Cherkessia were screened for 35delG mutation in the GJB2 gene (Karachays (N = 370), Cherkessians (N = 102), Russians (N = 35), Abazins (N = 137), Nogays (N = 122)).

**Results:** Population frequencies of 35delG mutation in Russians is 0.0143 (heterozygous carrier rate 1:35), in Karachais - 0.00135 (1:370), in Cherkessians - 0.0098 (1:51), in Abaza - 0.0145 (1:34), in Nogays - 0.0041 (1:122).

**Conclusion:** These differences result from origins and ethnogenesis of the peoples. Karachays belong to Caucasian anthropological type of the Balkan-Caucasian Caucasian race. They speak Karachai-Balkar language of Kipchak group of Turkic family. Nogays are much younger, time of its formation refers to the end of the XIV century. They are Turkified tribes of Mongol branch; speak one of the Turkic languages. Circassians and Abaza are Abkhaz-Adyge peoples; speaking Adyge languages of Abkhaz-Adyge language group. This work was partially funded by RFBR grants 14-04-00525, 15-04-01859.

#### E-P02.20

##### Oculocerebrorenal syndrome in children

**O. Marginean<sup>1,2</sup>, T. Marcovici<sup>1,2</sup>, D. Nicoara<sup>2</sup>, G. Brad<sup>1,2</sup>, O. Belei<sup>1,2</sup>, N. Mang<sup>1</sup>, S. Maruntelu<sup>2</sup>, M. Puiu<sup>1,2</sup>;**

<sup>1</sup>University of Medicine and Pharmacy „Victor Babes” Timisoara, Romania, <sup>2</sup>Louis Turcanu“Children’s Emergency Hospital, Timisoara, Romania.

**Introduction.** Oculocerebrorenal syndrome (Lowe syndrome) is caused by mutations in the OCRL-1 gene. It is a rare recessive X-linked disorder (1:500,000 people) characterized by eye, kidney and neurological impairment. Males are mostly affected. Cataracts and glaucoma are characteristic ocular findings in these patients and visual acuity may be impaired. Children with Lowe syndrome have delayed development, behavioral problems, feeding difficulties. The progressive kidney dysfunction can lead to chronic renal failure. Polyuria, dehydration, metabolic acidosis, electrolyte imbalance and osteopenia are present.

**Material and Methods.** We present a five years old boy with delayed development, protein-energy malnutrition, severe vitamin D deficiency and femur fracture. The case was evaluated by means of anamnesis, physical examination, paraclinical tests (imaging, functional, biology) and interdisciplinary check-ups.

**Results.** The patient exhibits typical facial appearance (elongated face, deep-set small eyes, frontal bossing), failure to thrive (BMI =12, below the 5th percentile for age and sex), congenital cataracts, corneal keloids, delayed motor and mental development, weak muscle tone, and self-injurious behavior. Proteinuria, aminoaciduria and albuminuria reveal renal tubular damage. Diagnosis of osteopenia was set by the presence of femoral fracture, severe vitamin D deficiency and confirmed through osteodensitometry.

Conclusions. The diagnosis was based on the particular phenotype association with biological abnormalities characteristic of the Lowe syndrome. Interdisciplinary management of the case is mandatory. Vital prognosis depends on the preservation of renal function.

#### E-P02.21

##### Technically difficult, diagnostically important - exon ORF15 of the RPGR gene in retinitis pigmentosa

**M. Oldak<sup>1</sup>, E. Ruszkowska<sup>1</sup>, S. Siwiec<sup>2</sup>, A. Pollak<sup>1</sup>, P. Stawiński<sup>1</sup>, K. Szulborski<sup>3</sup>, J. P. Szaflik<sup>3</sup>,**  
<sup>1</sup>Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland, <sup>2</sup>Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland, Warsaw, Poland, <sup>3</sup>Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland, Warsaw, Poland.

The aim of the study was to identify the genetic background of retinitis pigmentosa (RP) in a Polish family with previously excluded involvement of the majority of known RP genes except for the ORF15 exon in the RPGR gene (Xp21.1). ORF15 is a highly repetitive, purine-rich DNA region with a number of different polymorphic variants and thus difficult to study. Genomic DNA was isolated from peripheral blood of the family members (n = 9). Exon ORF15 was amplified in a long-range polymerase chain reaction (PCR) and sequenced using the new generation method (NGS). Presence of the identified variant was confirmed by direct Sanger sequencing of the amplicon encompassing the mutation. In the ORF15 region NM\_001034853:c.2899delG (p.E967Kfs\*122) mutation was detected. It completely segregated with the disease in the studied family. The identified change is pathogenic and has already been found to cause RP. It is estimated that more than a half of RPGR mutations is located in the ORF15 region. In families with a suspected X-linked inheritance of RP and in males with a negative family history of RP genetic tests should begin with analysis of ORF15.

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#### E-P02.22

##### GJB2 susceptibility mutations in a group of Romanian children with non-syndromic hearing loss

**C. Strugaru<sup>1</sup>, A. Brinzan<sup>2</sup>, R. Ursu<sup>1</sup>, C. Popa<sup>3</sup>, R. Bohilteu<sup>2</sup>, L. Bohilteu<sup>4</sup>,**  
<sup>1</sup>Department of Genetics, Bucharest, Romania, <sup>2</sup>Department of Pediatrics, Bucharest, Romania, <sup>3</sup>Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, <sup>4</sup>Department of Genetics, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

Background and aims: Sensorineural hearing loss (SNHL) is a multifaceted condition with profound medical, social, and cultural ramifications. For children born with moderate to profound hearing impairment, particularly in families in which there is no previous history of deafness, the diagnosis has a major impact on that family.

Objectives: Testing for mutations in GJB2, the gene for connexin 26 (Cx26), can account for up to 60% of prelingual nonsyndromic hearing impairment, is commercially available, and many more deafness-related genetic tests undoubtedly will follow in the near future.

Methods: In our study, we performed mutation screening for GJB2 in 42 non-syndromic hearing loss families, including those with cases of sporadic deafness. Peripheral blood lymphocyte DNA was used to amplify by polymerase chain reaction the Cx26 coding region, followed by 35delG mutation detection screening and complete sequencing. For DNA extraction we used QIAamp DNA Blood (QIAGEN), and PCR fragments were sequenced using the forward primer and the ABI BigDye Terminator v3.1 Cycle Sequencing Kit.

Results: The most frequent mutation was 35delG, followed by 167delT. In our study, we identified other mutations such: R32C, I20T, S19T and a compound heterozygous mutation 35delG/ R127H. However, a large fraction - 68% have only one mutant allele.

Conclusions: The high frequency of the 35delG mutation indicated its screening, regardless severity of hearing impairment or familial history. On the other hand, these results highlight the usefulness of 35delG mutation screening for genetic counseling and suggest the importance of entire sequencing of the gene responsible for DNFB1.

#### E-P02.23

##### Identification by next generation sequencing of novel compound heterozygous mutations in the CDH23 gene

**F. Cesca<sup>1</sup>, E. Bettella<sup>1</sup>, R. Pollr<sup>1</sup>, E. Leonardi<sup>1</sup>, B. Sicilian<sup>2</sup>, D. Croatto<sup>2</sup>, A. Murgia<sup>1</sup>,**

<sup>1</sup>Laboratory of Molecular Genetics of Neurodevelopment, Department of Women's and Children's Health, University of Padova, Padova, Italy, <sup>2</sup>Medical Center of Phoniatrics, Casa di Cura Trieste, Padua, Italy, Padova, Italy.

Hereditary sensorineural Non-Syndromic Hearing Loss (NSHL) is characterized by clinically indistinguishable phenotypes and genetic heterogeneity that up until recently have hampered effective early aetiological diagnosis. Next Generation Sequencing (NGS) technologies offer unprecedented diagnostic capacities and hold the promise of a significant expansion in knowledge on the pathogenic role of many disease-genes.

The CDH23 gene (NM\_022124) encodes a calcium dependent cell-cell adhesion glycoprotein, involved in stereocilia organization and hair bundle formation; its mutations, initially described as associated with Usher syndrome type 1D, are also responsible of autosomal recessive non-syndromic hearing loss.

By NGS strategy on Ion Torrent PGM platform, we have developed a multigenic NSHL panel which is integral in our NSHL molecular diagnostic pipeline. This NGS approach has lead to the identification of two CDH23 mutations in two sisters, aged 16 and 11, affected with congenital profound sensorineural non-syndromic hearing loss. No retinal alterations are present. The two girls had previously been found to carry a pathogenic GJB2 sequence variant.

The novel CDH23 variants: a missense mutation, c.6530C>A (p.P2177H), predicted as damaging by consent of in silico tools, and a splice-site mutation c.8966-1G>C, were both Sanger validated. Compound heterozygosity in the sisters was confirmed by mutation analysis in the normal hearing parents, who were found to be heterozygotes.

Our report, which describes two novel CDH23 mutations, provides evidence of the fact that truncating mutations of this gene, frequently found in Usher syndrome patients, may also be associated with non-syndromic sensorineural deafness.

#### E-P02.26

##### Targeted next generation sequencing identified a novel mutation in MYO7A causing Usher syndrome in an Iranian consanguineous pedigree

**D. Kooshavar, M. Razipour, M. Entezam, A. Karamzade, M. Saberi, M. Keramatipour, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.**

Introduction: Usher syndrome is mostly characterized by hearing impairment (HI) and adolescent-onset retinitis pigmentosa. It is categorized into two major types.

Materials and Methods: In this study a pedigree with two affected members were investigated. A 16 years old male with profound HI, rod-cone degeneration, and clinical diagnosis of Usher syndrome, result of a consanguineous marriage, was referred for genetic counseling/analysis. The proband had a one year old cousin, also result of a consanguineous marriage, with profound HI. Target region capturing of 13 Usher syndrome-related genes (CDH23, DFNB31, GPR98, MYO7A, PCDH15, USH1C, USH1G, USH2A, CLRN1, HARS, PDZD7, CIB2, and ABHD12) was performed followed by next generation sequencing (NGS).

Results: A novel homozygous variant in MYO7A gene was detected in the proband as NM\_000260:c.4513G>T(p.Glu1505\*). It was detected in homozygous state in the other affected member of the pedigree by Sanger sequencing. Segregation analysis was consistent with the AR pattern of inheritance. This variant was absent in population (1000G, ExAC, dbSNP) and disease-specific (ClinVar, OMIM, HGMD) databases, predicted to be disease-causing by multiple *in silico* predictive tools (mutation taster, SIFT, PolyPhen, CADD), and the substituted nucleotide was evolutionarily well conserved (ConSurf, phyloP, phastCons).

Conclusions: Based on the ACMG standards and guidelines for interpretation of sequence variants, it is classified as a pathogenic variant. Although MYO7A is the most common gene causing Usher syndrome typeI, NGS was able to detect this novel mutation. Therefore applying NGS in less investigated populations can still detect novel variants, even in well-studied genes for different disorders.

## E-P03 Internal organs & endocrinology (lung, kidney, liver, gastrointestinal)

### E-P03.01

#### Severe stage IV kidney disease in a child with ARID1B mutation : is there a link?

**K. Dahan<sup>1</sup>, S. Boulanger<sup>1</sup>, X. Lolin<sup>2</sup>, K. Ismaili<sup>2</sup>, A. Destré<sup>1</sup>**

<sup>1</sup>Centre de Génétique IPG, Gosselies, Belgium, <sup>2</sup>Service de Néphrologie Pédiatrique, HUDEF, Bruxelles, Belgium.

Segmental renal infarction is a rare condition in children, resulting from small arteries occlusion and responsible for renovascular hypertension. We report an unusual case of stage IV chronic kidney disease (CKD) with arterial hypertension in a 13-year-old boy with non-syndromic intellectual disability and a splice-site mutation in ARID1B. Nonenhanced CT shows multiple small (and larger) areas of renal parenchymal scarring, coexisting with normal appearing kidney. The renal sonography was normal at age 3.5. The cause of CKD remains unknown but multiple arteries occlusions and chronic ischemia are suspected. In absence of hypercoagulable factors or renal artery injury, this unusual presentation raises the hypothesis of a relationship between ARID1b and segmental renal infarction. Note the ARID1B gene was first identified in 2001 as an overexpressed transcription factor in vascular smooth muscle cells in response to kidney vascular injury.

### E-P03.09

#### Analysis of CYP21A2 gene mutations in patients from Ukraine with congenital adrenal hyperplasia

**S. Chernushyn<sup>1</sup>, L. Livshits<sup>1</sup>**

<sup>1</sup>Institute of Molecular Biology and Genetics, Kiev, Ukraine.

Congenital adrenal hyperplasia (CAH) refers to a group of disorders that arise from defective steroidogenesis. CAH is an autosomal recessive disorder with an incidence about 1:15,000. Using the long-range PCR, RFLP and ARMS methods, we have studied, deletion/conversion of CYP21A2, 8 bp deletion in exon 3 and eight CYP21A2 point mutations in 33 Ukrainian patients with different clinical signs of CAH. Mutations P30L, cluster exon 6 were not found. The analyzed mutations were detected in 32 from 33 CAH patients. The most prevalent mutation was deletion/conversion of CYP21A2, determined in 25 mutant alleles (37.9%), IVS2-13 A/C>G in 10 mutant alleles (15.2%), Q318X in 6 mutant alleles (9.1%), V281L in 6 mutant alleles (9.1%), R356W in 2 mutant alleles (3.0%) and I172N in 2 mutant alleles (3.0 %), E110Vfs in 2 mutant alleles (3.0 %). In rest 13 alleles (19.7 %) analyzed mutations were not detected. Interestingly, we observed higher Q318X frequency and lower I172N frequency in our population compared to the other European populations.

### E-P03.10

#### Significant phenotype variability of congenital central hypoventilation syndrome (CCHS) in three-generation family with polyalanine expansion mutation of PHOX2b gene

**E. Klásková<sup>1</sup>, M. Prochazka<sup>2</sup>, J. Drábel<sup>3</sup>, V. Curtisová<sup>2</sup>, J. Hyjánek<sup>2</sup>, V. Smolka<sup>1</sup>**

<sup>1</sup>Dpt. of Pediatrics, University Hospital and Medical Faculty of Palacky University, Olomouc, Czech Republic, <sup>2</sup>Dpt. of Medical Genetics, University Hospital and Medical Faculty of Palacky University, Olomouc, Czech Republic, <sup>3</sup>Institute of Molecular and Translational Medicine, University Hospital and Medical Faculty of Palacky University, Olomouc, Czech Republic.

Introduction: CCHS belongs to rare genetic disorders. It is characterized by hypoventilation secondary to absent responses to both hypercapnia and hypoxia being most pronounced during the sleep. CCHS has been shown to result from mutations in the PHOX2B gene located on chromosome 4p12.3. Materials and Methods: A rare familial case of CCHS caused by polyalanine expansion mutation of PHOX2B gene with significant phenotype variability in three generations. Case 1: A girl firstly admitted to the hospital with a history of severe cyanosis and respiratory failure related to pneumonia at six weeks of the age. Until four years of age, she was five times hospitalized because of respiratory failure in connection with respiratory tract infection. Case 2: a seemingly asymptomatic patient's father. Retrospectively we learned that he suffered from a severe headache and excessive daytime sleepiness. Both daughter and her father require overnight mechanical ventilatory support now. Case 3: Patient's grandmother who died after general anaesthesia at fifty years of age.

Results: Genetic testing revealed that the patient and her father are heterozygous for a polyalanine repeat expansion mutation of the PHOX2B gene (casual mutation c.741\_755dup15 in exon 3 of PHOX2B). Considering

grandmother's history, she is highly suspected of having CCHS as well. Conclusion: Although most CCHS PHOX2B mutations occur de novo, autosomal dominant inheritance with incomplete penetrance has been proposed. The increased attention should be paid to seemingly asymptomatic relatives as they are at increased risk of complications associated with respiratory infections, general anaesthesia, and drugs known to depress ventilation.

### E-P03.11

#### Characterization of inflammatory and apoptotic molecular markers of active Crohn's disease

**B. Stankovic<sup>1</sup>, N. Kotur<sup>1</sup>, B. Zukic<sup>1</sup>, S. Srzentic<sup>1</sup>, K. Klaassen<sup>1</sup>, S. Dragasevic<sup>2</sup>, D. Popovic<sup>2,3</sup>, A. Sokic-Milutinovic<sup>2,3</sup>, S. Lukic<sup>2,3</sup>, T. Alempijevic<sup>2,3</sup>, T. Milosavljevic<sup>2,3</sup>, G. Nikcevic<sup>1</sup>, S. Pavlovic<sup>1</sup>**

<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia, <sup>2</sup>Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia, <sup>3</sup>Medical faculty, University of Belgrade, Belgrade, Serbia.

Intestinal mucosal damage in Crohn's disease (CD) patients occurs as a result of deregulation of inflammatory and apoptotic processes. Transcriptional factor NF- $\kappa$ B has a key role in transcription of genes that mediate these events. The aim of this study was to elucidate molecular patterns that underlie these processes.

We analyzed expression levels of proinflammatory *IL-6* and *TNF- $\alpha$* , and apoptotic *Bcl-2*, *Bax*, *Fas* and *FasL* genes in intestinal mucosa and peripheral blood mononuclear cells (PBMC) of 24 patients with active CD and 21 controls, using qRT-PCR methodology. Among these participants, we selected 10 CD patients and 5 controls in order to conduct EMSA analysis of the DNA binding activity of NF- $\kappa$ B from the nuclear extracts of donors' intestinal mucosal samples.

Results showed that expression levels of *IL-6* and *TNF- $\alpha$*  were significantly increased, while expression level of *Bcl-2* was significantly decreased in ileal inflamed mucosa of CD patients. Our results also revealed that the expression level of *FasL* in PBMC of male CD patients was significantly decreased. The analysis of the DNA binding activity of NF- $\kappa$ B revealed an association of decreased level of NF- $\kappa$ B binding activity and increased expression level of *TNF- $\alpha$*  with intestinal mucosal fragility.

Our results demonstrated that expression profiles of selected proinflammatory and apoptotic genes, as well as the DNA binding activity status of NF- $\kappa$ B could be considered as molecular markers of active CD.

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### E-P03.12

#### Cys282Tyr, His63Asp and Ser65Cys mutations in the diagnosis of hereditary hemochromatosis type 1

**S. Turyk<sup>1</sup>, M. Sakurai<sup>2</sup>, S. Bonsergent<sup>2</sup>, M. Notaris<sup>2</sup>, O. Rabinovich<sup>2</sup>, N. Fernandez<sup>2</sup>, J. Benavidez<sup>2</sup>, K. Smith<sup>2</sup>**

<sup>1</sup>Hospital Britanico de Buenos Aires, Buenos Aires, Argentina.

Hereditary hemochromatosis type 1 (HH) is an autosomal recessive disorder of iron metabolism. It is characterized by progressive iron overload and caused by mutation in the HFE gene on chromosome 6. The predominant feature of HH is excessive absorption of dietary iron and its deposition in parenchymal tissues and results in cirrhosis, diabetes, skin pigmentation and testicular failure. Prevalence is estimated at 1 in 200 to 1 in 2000.

26 HH patients ( 9 females and 17 males) and 72 healthy controls were screened for the Cys282Tyr, His63Asp and Ser65Cys , using polymerase chain reaction amplification of genomic DNA , followed by digestion with Rsa 1 and Bel-1. All patients had the following parameters: iron studies including serum Fe, ferritin and transferrin saturation, serology for hepatitis B and C, liver function tests and abdominal echography .

The mean age at genotype diagnosis was 55 years in males and 52.8 years in females. 23 from 26 (88.46%) HH patients were homozygous for Cys 282Tyr mutation . Two (7.7%) were compound heterozygous for Cys 282Tyr/His63Asp and one (3.84%) was for Cys282Tyr/Ser65Cys. Five (6.9%) of our controls were heterozygous for Cys282Tyr and one (1.3%) was heterozygous for His63Asp.

Hereditary hemochromatosis type 1 is an underdiagnosed disorder. The most frequent form is associated with homozygosity of the Cys282Tyr mutation. Because of the late manifestation of HH, it should be considered in any patient with dysfunction of parenchymal tissues.

## E-P03.14

### The spectrum of the most common CFTR gene mutations at Hospital of Lithuanian University of Health Sciences Kaunas Clinics during the years 2003-2015

I. Poceviciene, I. Valanciute, B. Sabaniene, V. Asmoniene;

Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Kaunas, Lithuania.

**Introduction:** This study aims at reviewing the patients tested for CFTR gene mutations at Hospital of Lithuanian University of Health Sciences Kaunas Clinics and to find most common CFTR gene mutations of the patients tested.

**Materials, methods:** 355 patients were tested for CFTR gene mutations in the period of 2003-2015 in the laboratory of Genetics and Molecular Medicine at Hospital of Lithuanian University of Health Sciences Kaunas Clinics. CFTR gene mutations were tested using commercial INNO-LiPA CFTR19 (Innogenetics, Belgium) hybridisation kit from 2003 till 2010, xTAG® Cystic Fibrosis 39 Kit v2 (Luminex) from 2011 till 2013 and xTAG® Cystic Fibrosis 71 Kit v2 (Luminex) for the 71 CFTR mutations from 2013 till 2015.

**Results:** The CFTR gene mutations were found in 17,5% of all analyzed cases. The most common mutation, as expected, was F508del with a incidence of 63,86 % out of all mutations detected. As far as F508del is concerned, 14 patients were diagnosed to be homozygotes (508del/508del) and 25 heterozygotes. 9 patients were diagnosed to have CFTRdele2,3 (21kb) mutation that accounts for 10,84% of all detected. R553X mutation showed the incidence of 10,84% (9 patient). 6 patients were diagnosed with G542X mutation (7,23%), 2 patients with R117H mutation (2,41%), 3 patients with 3849+10kb C>T mutation (3,61%). 1 patient with R1066C mutation (1,20%). 10 patients were diagnosed to have compound heterozygote genotypes : R553X/CFTRdele2,3 (3 patients), G542X/R553X (6 patients), F508del/CFTRdele2,3 (1 patient).

In conclusion, the spectrum of CFTR gene mutations was similar to that reported for European population.

## E-P03.15

### Rare mutations in CFTR gene. The difficulty of genotype-phenotype association in a Spanish family

J. Porta<sup>1</sup>, J. M. Porta<sup>1</sup>, S. Franco<sup>1</sup>, E. Perez-Ruiz<sup>2</sup>, V. Perez-Valero<sup>2</sup>, C. Benito<sup>2</sup>;

<sup>1</sup>Genologica, Malaga, Spain, <sup>2</sup>Regional Hospital Laboratory of Malaga, Malaga, Spain,

<sup>3</sup>Section on Pediatric Pulmonology, Regional Hospital Laboratory of Malaga, Malaga, Spain.

**Introduction:** Cystic fibrosis (CF) is a genetic disorder characterized by the production of sweat with a high salt content and mucus secretions with an abnormal viscosity. Cystic fibrosis is an autosomal recessive disease, most common among Caucasian children. It is caused by mutations in CFTR gene which plays a role in the regulation of transmembrane hydroelectrolytic flux. The most common form of cystic fibrosis is associated with respiratory symptoms, pancreatic insufficiency, etc. There is considerable genotype-phenotype association but the rare mutations in CFTR gene hampers this objective.

**Case:** A 9-month-old girl with positive neonatal screening for cystic fibrosis (on the basis of sweat test results chloride concentration of 79 mmol/L) must be confirmed by identification of CFTR gene mutations.

Recurrent mutation CFTR panel was firstly analyzed. Traditional but precise Sanger DNA sequencing were used to search for specific mutations in the CFTR gene followed by MLPA CFTR gene deletion insertions variants.

Early detection of pancreatic insufficiency is essential to optimize health and outcomes in cystic fibrosis patients. Faecal elastase and other test were employed to measure pancreatic insufficiency. The results were associated with pancreatic insufficiency.

The research participants were the mother, the father and the paternal uncle and paternal aunt besides two brothers of the affected girl.

**Results and Discussion:** The genetic test detected two described probably pathogenic variants c.2988+1Kbde18,6Kb and c.711+5G>A in heterozygosity and several polymorphism in the patient sample. This discussion describes the possible genotype-phenotype association between of these mutations, variants, polymorphisms, population and clinical data.

## E-P03.16

### The challenge of diagnosis of cystinuria

S. Vorslova, Z. Krumina, Z. Daneberga, I. Nartisa, P. Vevere;

Children Clinical University Hospital, Riga, Latvia.

Cystinuria is an autosomal recessive disease that can be classified into three subtypes: Type A involves the mutation of both alleles of SLC3A1 (2p21); heterozygotes exhibit a normal amino acid urinary pattern, Type B involves

the mutations of both alleles of SLC7A9 (19q13.11); heterozygotes typically exhibit increased cystine and dibasic amino acid urinary excretion, and Type AB is caused by one mutation in SLC3A1 and one mutation in SLC7A9; this mixed-type may be caused by the interaction of two distinct mutant genes. The prevalence is 1 in 7,000.

We report data of two brothers with similar clinical and laboratory findings born in a non-consanguineous family. The eldest brother was two years old at the time of genetic consultation, and the youngest brother was four months old. The brothers presented severe sharp spasmotic pain in the backs, sides, and abdominal areas. We measured markedly increased concentrations of protein and red blood cells in the brothers' urine. Amino acid analysis revealed an elevated concentration of urinary cystine and dibasic amino acid. An ultrasonographic investigation revealed the formation of kidneys stones in the eldest brother.

Mutation analysis of the SLC3A1 and SLC7A9 genes revealed that the eldest brother was a heterozygous carrier for mutation c.241C>T, exon 1 of the SLC3A1 gene; no mutations were found in the youngest brother. The genetic cause of the clinical findings in both patients remains unexplained. The differences in the genotypes of the brothers may suggest the presence of unknown genetic variations responsible for the siblings' clinical symptoms.

## E-P03.18

### Case report: 46,XX testicular disorders of sex development

Y. Shecherbak;

Center of medical genetics, National specialized children hospital, Kyiv, Ukraine.

**Introduction:** The XX-testicular disorders of sex development are characterized by disparity between female chromosomes and male phenotype. At first XX-form disorder has been described as a XX-male syndrome (syndrome de la Chappelle). In 85% of cases disorder due to the presence in the genome gene SRY (hidden translocation fragment Yp11 on chromosome X or on autosomes, or hidden chromosomal mosaicism). Was described SRY-positive and SRY-negative (mutations in the genes SOX3, SOX9 and other) forms.

**Materials and methods:** The retrospective analysis of 150 genetic cards of children with disorders of sex development was carried out. During the period 1999-2015 y. the 10 cases of XX- sex inversion was diagnosed. Patients were examined using molecular cytogenetic analysis with locus-specific probes CEP X and SRY.

**Results:** The patient's age at the time of the first consultation ranged from 6 months to 18 years. Statements were associated with abnormal external genitalia and the necessity to confirm the appropriateness of the chosen civil gender, or presence of cryptorchidism, hypogonadism and gynecomastia. Were described clinical cases with SRY-positive and SRY-negative 46,XX testicular disorder.

**Conclusions:** Only cooperation experts and using genetic analysis allow verifying the diagnosis and making gender selection optimally correct for patient. In the case of abnormal external genitalia newborn when deciding his gender should focus not only on karyotype because it does not always coincide with gonadal, hormonal and phenotypic sex.

## E-P03.19

### Gene polymorphisms of eNOS 4a/4b and eNOS G894T in young nephrotic syndrome patients

F. Tripont<sup>1</sup>, A. G. Crauciuc<sup>1</sup>, A. Boglis<sup>1</sup>, C. Duicu<sup>1,2</sup>, C. Banescu<sup>1,2</sup>;

<sup>1</sup>University of Medicine and Pharmacy Tîrgu Mureş, Tîrgu Mureş, Romania, <sup>2</sup>Clinical and Emergency Hospital, Tîrgu Mureş, Romania.

**Introduction:** Due to the fact that nitric oxide plays a role in regulation of renal sodium handling, vascular resistance and sympathetic activity we designed this study to investigate if there is any correlation between eNOS 4a/4b and eNOS G894T genes polymorphisms and nephrotic syndrome (NS) in young patients from Transylvania, Romania.

**Material and methods:** We performed a case-control study, approved by the ethic committee of the University of Medicine and Pharmacy Tîrgu Mureş (no.113/14.12.2015). Control group comprised of 100 children while patients group consisted of 68 NS cases. Genomic DNA was amplified using specific primers and technique.

**Results:** The mean age for both groups was 6.2 years  $\pm$ 3.1 standard deviation. According to the sex ratio 55% were boys and 45% girls with an approximated equally distribution between the groups. The genotype frequencies for eNOS 4a/4b in control group were 2% 4a/4a, 37% 4a/4b, 61% 4b/4b while in patients group were 13.23% 4a/4a, 47.05% 4a/4b and 39.72% 4b/4b. The frequencies of eNOS G894T genotypes in the control group were 56% GG, 18% GT, 24% TT while in patients group were 46.4% GG, 44.7% GT and 8.9% TT. From the patients with 4a/4a genotype 66.66% of them

were homozygotes variant TT for eNOS G894T while the remaining were heterozygotes.

Conclusion: The presence of eNOS 4a allele represent a risk factor for developing NS (p=0.007). The eNOS 894T allele is not associated with NS but in the case of combined variant genotype it is possible to play a role in predisposition to NS.

#### E-P03.20

##### An ARHGAP24 homozygous missense variant in a child with focal segmental glomerulosclerosis and end stage renal disease

A. Mory<sup>1</sup>, D. Magen<sup>2,3</sup>, T. Paperna<sup>1</sup>, A. Kurolap<sup>1</sup>, H. N. Baris<sup>1,3</sup>,

<sup>1</sup>The Genetics Institute, Rambam Health Care Campus, Haifa, Israel, <sup>2</sup>Pediatric nephrology institute, Ruth Rappaport Children's Hospital, Rambam Health Care Campus, Haifa, Israel, <sup>3</sup>Rappaport School of Medicine, Technion – Israel Institute of Technology, Haifa, Israel.

Introduction: Focal segmental glomerulosclerosis (FSGS) is a clinicopathologic syndrome, associated with proteinuria and progressive renal failure. In the pediatric age group, primary FSGS usually underlies steroid resistant nephrotic syndrome, and is caused by both idiopathic and genetic causes. We present a 10 year-old boy, born to healthy Muslim Arab first-degree cousins, who was referred to the Pediatric Nephrology Institute with end stage renal disease secondary to FSGS. Nephrotic syndrome was suspected and genetic testing was pursued.

Methods: The TruSight™ One panel, was employed on Illumina's Next-Seq500. Analysis focused on variation in candidate genes, followed by filtering rare variants (<0.05%). We prioritized the variants using the VarElect tool with relevant clinical search terms.

Results: No pathogenic variants were detected in candidate genes, mainly NPHS1, NPHS2, and WT1. Further analysis revealed a homozygous variant in the ARHGAP24 gene, NM\_001025616.2:c.1442C>T; p.T481M. This variant was previously implicated in association with FSGS.

Discussion: The ARHGAP24 gene encodes a RhoA-activated Rac1 GTPase-activating protein, which plays a role in differentiating podocytes and cell adhesion. A mutation in ARHGAP24 has been reported in association with autosomally dominantly inherited familial FSGS, while here we report for the first time a homozygous variant in a child with renal failure. In-silico pathogenicity prediction tools were contradicting; 122 heterozygote alleles, but no homozygotes, were observed in the Exom Aggregation Consortium (ExAC) database. Further studies are needed in order to establish the role of a biallelic mutation, in general, and the homozygous p.T481M mutation, in particular, in the pathogenesis of hereditary FSGS.

#### E-P03.22

##### Results from GWAS studies regarding insulin resistance - usefulness in the clinical setting

A. Chirita-Emandi<sup>1</sup>, N. Andreescu<sup>1</sup>, S. Farcaș<sup>1</sup>, I. Velea<sup>2</sup>, M. Puiu<sup>2</sup>,

<sup>1</sup>University of Medicine & Pharmacy Victor Babes, Genetics Department, Center of Genomic Medicine, Timisoara, Romania, <sup>2</sup>University of Medicine & Pharmacy Victor Babes, Paediatrics Department, Timisoara, Romania, <sup>3</sup>University of Medicine & Pharmacy Victor Babes, Genetics Department, Center of Genomic Medicine, Regional Centre of Medical Genetics Timis, Emergency Hospital for Children "Louis Turcanu", Timisoara, Romania.

Background: Obesity is at epidemic levels worldwide. Insulin resistance, one of its complications, could be prevented/addressed through diet and medication. Therefore determining, at young age, which obese patients are predisposed to develop insulin resistance is important for clinical practice. Aim: To provide a study design that would translate the findings from GWAS studies regarding insulin resistance into a model with high predictive value for use in the clinical setting.

Method: We reviewed the literature for GWAS studies regarding insulin resistance, and evaluated the potential cost and benefit for a genetic model involved in insulin resistance.

Results: Over 250 loci are significantly and robustly associated with type 2 diabetes(T2D) and/or obesity-related traits. However, less than 20 loci are directly associated with HOMA-IR in adults. For a robust predictive model, sample size needs to be larger than 2500. With a cross-sectional design, the price for clinical/metabolic assessment and genotyping is significant. Importantly, some studies replicating GWAS results on insulin resistance, have found only a slight increase in predictive ability of a model(using regression/neuronal networks) after adding the genetic signature specific for insulin resistance, while the BMI provided the highest predictive value.

Conclusion: Presently, clinical utility for prognosis of common variants whose allele frequencies are statistically correlated with insulin resistance is not established. Although, replication studies are costly, we plan to develop such a study to assess the improvement in significance of risk stratification, in

our Genomic Centre.

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#### E-P03.23

##### Clinical outcomes of gastroduodenal diseases in Yakut patients from Eastern Siberia: iceA1 strains of Helicobacter pylori associated with early onset of chronic gastritis

N. N. Gotovtsev<sup>1</sup>, N. A. Barashkov<sup>1,2</sup>, K. E. Savvinova<sup>2</sup>, M. V. Pak<sup>3</sup>, M. P. Alexeeva<sup>3</sup>, V. G. Pshehnikova<sup>2</sup>, A. M. Rafailov<sup>2</sup>, S. N. Lekhanova<sup>2</sup>, S. A. Fedorova<sup>1,2</sup>,

<sup>1</sup>Yakut Scientific Center of complex medical problems, Yakutsk, Russian Federation, <sup>2</sup>MK Ammosov North-Eastern Federal University, Yakutsk, Russian Federation, <sup>3</sup>Republican hospital №1 – National Center of Medicine, Yakutsk, Russian Federation.

Clinical outcome of Helicobacter pylori (Hp) infection is associated with virulence genotypes. The iceA gene of Hp have been reported to be associated with peptic ulcer, the importance of iceA on clinical outcomes is controversial [Shiota et al., 2010]. The iceA gene has two alleles iceA1 and iceA2 [Peek et al., 1998]. Until now, allele frequency of iceA Hp circulating in Eastern Siberia (Sakha Republic) is unexplored. We studied Hp DNA samples extracted from biopsies of 92 Yakut patients with gastroduodenal diseases, 43 of them were adolescents (mean age 15.05 ± 4.11 years) and 49 were adults (mean age 43.45 ± 12.42 years). The iceA1 was identified in 65.2% and iceA2 in 34.7% of cases (60 and 32 patients, respectively). We did not find associations of iceA1/iceA2 with erosions and ulcers (p>0.05), sex of patients (p>0.05), and place of birth or residence (urban and rural) (p>0.05). We found association between iceA1 and age of patients. The iceA1 allele was found among adolescent patients more frequently (79%) than among adult patients (53%) (p<0.01). Similar association was found in Tunisian patients with gastroduodenal diseases [Mansour et al., 2010]. In other reports of such kind associations with age was not performed. Thus, we suggest that iceA1 may be associated with early onset of chronic gastritis. This work was supported by the #6.656.2014/K project and Grant the head of the Sakha Republic (Yakutia) for young scientists, specialists and students in 2016 (February 8, 2016, #105-RG).

#### E-P03.24

##### GWAS and HLA genotyping-based association analysis for hepatitis B virus-related chronic hepatitis and hepatocellular carcinoma

K. Tokunaga<sup>1</sup>, H. Sawai<sup>1</sup>, N. Nishida<sup>1,2</sup>, H. Miyadera<sup>1,2</sup>, M. Mizokami<sup>2</sup>,

<sup>1</sup>Dept Human Genetics, Grad Sch Medicine, Univ Tokyo, Tokyo, Japan, <sup>2</sup>Res Cent Hepatitis Immunol, Natl Cent Global Health Med, Ichikawa, Japan.

SNP-based genome-wide association studies (GWAS) performed in different ethnic groups, including our GWAS in the Japanese population, have consistently indicated that HLA-DPA1/DPB1 is the strongest susceptibility region to hepatitis B virus (HBV)-related chronic hepatitis (CHB). Follow-up studies based on genotyping of HLA-DPA1 and HLA-DPB1 showed multiple susceptible and protective alleles/haplotypes to CHB in Japanese, Chinese, Korean, and Thai populations. Moreover, additive effects of these susceptible or protective alleles on odds ratios were observed. Interestingly, heterozygotes of DPB1 protective and susceptible alleles were significantly associated with protection, indicating that one protective HLA-DPB1 molecule can provide dominant protection. Binding assays between susceptible or protective HLA-DP proteins and Hepatitis B virus-derived peptides detected specific peptides. Next, we expanded HLA genotyping-based association analyses to the other HLA class II genes, HLA-DQA1, DQB1 and DRB1. Unexpectedly, some DRB1-DQA1-DQB1 haplotypes clearly showed stronger associations than DPA1-DBP1 haplotypes. Furthermore, GWAS and HLA genotyping-based association analysis on HBV-related hepatocellular carcinoma (HCC) identified new protective alleles in HLA class I genes in addition to previously identified HLA-DPB1 protective allele. Since many diseases have been reported to be strongly associated with the HLA region by means of SNP-based GWAS, careful association studies based on HLA genotyping are essential to identify the primary susceptibility variants in this particular genome region.

**E-P03.25****Association of peroxisome proliferator-activated receptor-gamma polymorphisms with inflammatory bowel disease in a Hungarian cohort.**A. Penyige<sup>1</sup>, S. Poliska<sup>2</sup>, L. Nagy<sup>3</sup>;<sup>1</sup>Univ. of Debrecen, Faculty of Medicine, Dept. of Human Genetics, Debrecen, Hungary,<sup>2</sup>Univ. of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Debrecen, Hungary, <sup>3</sup>Univ. of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Debrecen, Hungary.

**Introduction:** Inflammatory bowel diseases (IBD) show increasing incidence in the last few years in Hungary. Since genetic susceptibility of patients plays an important role in the pathogenesis of the disease, it is important to identify new susceptibility genes. Peroxisome proliferator-activated receptor gamma (PPARG) is expressed in the colon and has protective effects against inflammatory processes. Our aim was to examine the association of four polymorphisms of PPARG in a well-characterized Hungarian IBD cohort.

**Material and Methods:** 575 Crohn's disease (CD), 103 ulcerative colitis (UC) patients and 486 sex and age matched controls were examined in our study. Four polymorphisms of PPARG [rs10865710 (C-681G), rs2067819, rs3892175 and rs1801282 (Pro12Ala)] were genotyped by TaqMan genotyping assays.

**Results:** The Pro12Ala polymorphism showed significant association with CD when the frequencies of the homozygous variants (Pro/Pro vs. Ala/Ala) were compared. The minor Ala/Ala genotype was significantly less frequent in CD patients compared to the controls (OR= 0.33; 95%CI= 0.12-0.94; P= 0.03), suggesting a potential protective effect of the Ala allele. GAGG haplotype of PPARG also confers protective effect in CD (OR= 0.72, 95% CI: 0.53-0.97; P= 0.028) and in UC (OR= 0.14; 95% CI: 0.05-0.42; P= 3.78 × 10-5) too. While GAGC increases the risk of UC (OR= 6.70; 95% CI: 3.41-13.17; P= 3.85 × 10-10).

**Conclusions:** In the present study we demonstrated a significant association between PPARG polymorphisms and the development of CD and UC at single loci level and also in haplotype combinations.

**E-P03.26****Gross deletion of GHR in a patient with Laron Syndrome**A. Arman<sup>1</sup>, H. Simsek<sup>1</sup>, G. Y. Mutlu<sup>2</sup>, H. K. Bekmez<sup>2</sup>, S. Cici<sup>2</sup>, S. Hatun<sup>1</sup>;<sup>1</sup>The Department of Medical Genetics, Marmara University Medical School, Istanbul, Turkey, <sup>2</sup>The Department of Pediatric Endocrinology, Zeynep Kamil Education and Research Hospital, Istanbul, Turkey, <sup>3</sup>The Clinics of Children Disease, Zeynep Kamil Education and Research Hospital, Istanbul, Turkey, <sup>4</sup>The Department of Pediatric Endocrinology and Diabetes, Kocaeli University Medical School, Istanbul, Turkey.

**Introduction:** Laron syndrome (LS) is an autosomal recessive disorder characterized by severe postnatal growth failure, short stature, normal or increased plasma growth hormone (GH), and low levels of insulin like growth factor-1 (IGF-1) and IGF binding protein 3 (IGFBP-3). These characteristics result from defective GH receptor (GHR) function and, thus, a GH insensitive state. We analyzed the GHR gene for mutations and polymorphisms in a patients with Laron-type dwarfism.

**Materials and Methods:** Patients were selected in this study based on their clinical and laboratory characteristics. Genomic DNA was isolated from bloods of LS children according to salting out method. Exon 2-10 specific PCRs and their flanking splice sites were amplified by polymerase chain reaction (PCR) using the primers. PCR products were visualized on 2% agarose gels.

**Results:** PCR results showed that GHR from patient was deleted from exon 4-10 and this deletion causes defective GHR missing intracellular domain, transmembrane domain and large part of extracellular domain (exon 4-7). **Conclusions:** We identified gross deletion (exon 4-10) from GHR gene in patient with Laron and this is second report for deletion of exon 4-10 of GHR in patient with laron syndrome.

Yamamoto H1, Kouhara H, Iida K, Chihara K, Kasayama S. A novel growth hormone receptor gene deletion mutation in a patient with primary growth hormone insensitivity syndrome (Laron syndrome). Growth Horm IGF Res. 2008 Apr;18(2):136-42.

**E-P03.27****A novel single variant in MEFV gene causing Mediterranean Fever and Behcet disease: case report of Moroccan girl**M. Zerkaoui<sup>1</sup>, F. Z. Laarabi<sup>2</sup>, Y. Ahjoun<sup>3</sup>, B. Chkrire<sup>3</sup>, A. Sefiani<sup>1,2</sup>;<sup>1</sup>Centre de Génétique Humaine, Faculté de Médecine et Pharmacie de Rabat.<sup>2</sup>Mohammed V University in Rabat, Rabat, Morocco, <sup>3</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, <sup>3</sup>Service de Pédiatrie IV, Hôpital d'Enfant, Mohammed V University in Rabat, Rabat, Morocco.

Familial Mediterranean Fever (FMF) is an autoinflammatory disease of

unknown etiology, characterized clinically by recurrent attacks of sudden-onset fever with arthralgia and/or thoracoabdominal pain, and pathogenetically by autosomal recessive inheritance due to a mutation in the MEFV gene. Behcet's disease (BD) is an inflammatory disease characterized with recurrent oral and genital aphthous ulcerations, uveitis, and skin lesions. It has been reported that the MEFV gene mutation responsible for FMF is probably a susceptibility factor for BD, particularly for cases with vascular involvement, and both disorders can occur concurrently in a subject, as in this case. Herein, we present a case of Moroccan girl with FMF and BD coexistence. We analyzed the entire MEFV gene after exclusion of recurrent mutations in exons 2 and 10. Molecular testing revealed a novel single variant c.2078T>A (p.Met693Lys) that could be responsible of the association of FMF and BD.

**E-P03.28****Application of Urinary C-peptide to Creatinine Ratio (UCPCR) for Discrimination of Maturity Onset Diabetes of the Young (MODY) in the Emirati Population**H. Daggag<sup>1</sup>, A. Buckley<sup>1</sup>, N. Lessan<sup>1</sup>, A. Al-Tikriti<sup>1</sup>, K. Colclough<sup>2</sup>, S. Ellard<sup>2</sup>, M. Taysir Barakat<sup>1</sup>;<sup>1</sup>Imperial College London Diabetes Centre, Abu Dhabi, United Arab Emirates, <sup>2</sup>Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom.

It has become more challenging to distinguish between type 1, type 2 and monogenic diabetes using the standard parameters such as age, BMI and absence of islet cell or GAD antibodies. Besser et al. (2011) and Besser et al. (2013) have shown distinction is possible by utilising UCPCR.

We examine the clinical utility of current screening criteria and UCPCR testing in the Emirati population. Paediatric and adult cases are recruited and 2 hour post-prandial UCPCR is determined. Next generation sequencing of known monogenic diabetes genes is performed to confirm a diagnosis of MODY and to help identify patients for the investigation of novel genetic aetiologies in the Emirati population, by performing whole exome sequencing. Recruited patients were 126 type 1 diabetes and 16 type 2 diabetes paediatric patients and 67 type 1 diabetes and 64 type 2 diabetes adult patients. 2 Tailed Spearman correlation test showed that in type 1 diabetes patients, UCPCR was negatively correlated with duration of diabetes ( $r=-0.6$ ,  $p < 0.001$ ); there was a weak correlation between UCPCR and duration of diabetes in patients with type 2 diabetes ( $r=-0.23$ ,  $p = 0.044$ ). UCPCR was significantly lower in paediatric patients with type 1 diabetes with duration of less than 1 year (median 0.583) compared to more than 1 year (median 0.015) ( $p < 0.001$ ).

We suggest that UCPCR test could be valuable for discrimination between type 1 and type 2 diabetes and/or MODY in paediatric and adult Emirati patients and can lead to an earlier clinical diagnosis and adequate management.

**E-P03.29****Exome analysis establishes the diagnosis of microvillus inclusion disease (MVID) in a family with cholestatic liver disorder as the predominant clinical feature**D. Bartholdi<sup>1</sup>, C. Courage<sup>2</sup>, C. Margini<sup>3</sup>, F. Dallèves<sup>4</sup>, J. Dufour<sup>3</sup>, S. Gallati<sup>1</sup>;<sup>1</sup>Division of Human Genetics, University Children's Hospital, Bern, Switzerland,<sup>2</sup>Folkhälsoinstitutet of Genetics, Helsinki, Finland, <sup>3</sup>Department of Visceral Surgery and Medicine, University Hospital, Bern, Switzerland.

Microvillus inclusion disease (MVID) is a severe congenital enteropathy characterized by intracytoplasmatic microvillus inclusions and brush border atrophy in intestinal epithelial cells. It is an autosomal recessive disorder caused by mutations in the MYO5B gene (MIM#606540) encoding the myosin Vb protein. The disorder is characterized by intractable secretory diarrhoea in infancy. Intestinal failure secondary to diarrhoea is frequent and children with MVID are dependent on parenteral nutrition. Long-term outcome is generally poor.

We report a patient who is now 30 years of age and who presented with unclassified cholestatic liver disorder and recurrent diarrhoea four weeks after birth. His two older brothers had died of an unclassified liver disorder in infancy. The clinical course in the patient reported here stabilized in the second year of life after which he suffered from occasional episodes of diarrhoea only, but presented with severe cholestatic liver disorder as the predominant clinical feature. The putative diagnosis of progressive familial intrahepatic cholestasis (PFIC1/2) was established. However, molecular analysis of the ATP8B1 and ABCB11 genes did not reveal any pathogenic mutations, excluding PFIC1/2 as the likely diagnosis. In order to establish the correct diagnosis we performed exome sequencing. This analysis revealed two heterozygous disease-causing mutations in the MYO5B gene

(c.242A>G, p.His81Arg and c.4798C>T, p.Gln1600\*) establishing microvillus inclusion disease (MVID) as the diagnosis in the patient. In summary, we present further evidence for the broad clinical variability in MVID and confirm that this disorder can manifest predominantly as cholestatic liver disorder.

#### E-P03.30

#### A panel of five gene polymorphisms investigated in childhood nephrotic syndrome in Romania

C. Duicu<sup>1</sup>, C. O. Marginean<sup>1</sup>, F. Tripon<sup>2</sup>, A. Crauciuc<sup>2</sup>, C. Banescu<sup>2</sup>;

<sup>1</sup>Pediatric Department No 1, University of Medicine and Pharmacy Tg. Mures, Romania, <sup>2</sup>Tg. Mures, Romania, <sup>2</sup>Genetic Department, University of Medicine and Pharmacy Tg. Mures, Romania, Tg. Mures, Romania.

**Background:** Nephrotic syndrome (NS) is a common renal problems encountered in children.

Nephrotic syndrome (NS) is caused by different disorders that damage the kidneys. Recent studies have described that certain genetic mutations are involved in the NS pathway, prognostic and response to therapy.

**Aim:** This study was performed to investigate if there is any association of some polymorphisms (ACE angiotensin converting enzyme I/D gene polymorphism, VEGF I/D (vascular endothelial growth factor), NPHS2, Tumor Necrosis Factor  $\alpha$  (TNF  $\alpha$ ) G308A and IL-6 174G>C gene polymorphism and nephrotic syndrome.

**Materials and methods:** The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy Tg. Mures. Our study included a number of 57 patients with NS and a control group which included 123 healthy persons. The gene polymorphisms were determined by the PCR and PCR-RFLP techniques using specific primers.

**Results:** Mutations (NPHS2) were detected in 100% of congenital-onset NS. Patients with NS had a higher percentage of II and ID genotype than the control group (especially in girls).

**Conclusions:** We were unable to show a relationship between VEGF I/D, TNF $\alpha$  G308A, IL-6 174G>C gene polymorphism and NS. There is an association between NS and ACE I/D polymorphism, the deletion might be a protective factor and insertion a risk factor. Mutation in NPHS2 gene represent a risk factor for congenital NS.

Further investigations with a larger scale, multicenter studies are necessary to confirm/clarify these findings and to elucidate the role of these genes polymorphisms in NS development and course of treatment.

#### E-P03.31

#### Molecular screening of the human Melanocortin 4 Receptor (MC4R) gene in obese Maltese Type 2 Diabetic patients

S. Eljali, N. P. Pace, J. Vassallo, A. E. Felice;

University of Malta, Msida, Malta.

Obesity is a complex trait arising from complex gene-lifestyle interactions. It is a risk factor for cardio-metabolic diseases, including type 2 diabetes (T2DM). Investigations on early onset/severe obesity have identified variants in genes acting on the central regulation of appetite. Of particular interest is the melanocortin 4 receptor (MC4R). Mutations in this gene represent the most frequent cause of early-onset non-syndromic obesity.

The aim of this investigation was to perform mutational screening of the MC4R exon in obese Maltese T2DM patients.

#### Methods

We sequenced the MC4R exon in 192 obese T2DM patients of Maltese ethnicity. PCR amplification was followed by Sanger sequencing at GATC Biotech, Germany.

#### Results

MC4R sequence variants are uncommon in obese T2DM patients. Sequence electropherograms were screened for homozygous SNPs and insertion-deletion variants (InDels). No homozygous SNPs or indels were detected in the study

cohort. Sequence electropherograms were inspected to identify heterozygous variants in the MC4R exon. Five cases (3 Males, 2 Females) were identified carrying the missense rs2229616 variant in the heterozygous state. rs2229616 C/T polymorphism results in a Valine to Isoleucine substitution at codon 103. Our data shows that MC4R mutations are rare, and do not contribute to adult obesity associated with insulin resistance in T2DM. These findings are in keeping with studies that showed that obesity causing mutations in MC4R have a very low prevalence in an obese cohort from Southern Italy.

#### Conclusion

This is the first investigation into the prevalence and spectrum of MC4R variants in obese Maltese adults.

#### E-P03.32

#### PRSS1, SPINK1, CFTR, and CTRC mutations in Korean patients with idiopathic pancreatitis

S. Cho, S. Shin, K. Lee;

Yonsei university, Seoul, Korea, Republic of.

**AIM:** This study aimed to identify mutations in PRSS1, SPINK1, CFTR and CTRC genes with idiopathic pancreatitis in Korean patients.

**METHODS:** The population under study consisted of 116 Korean subjects (65 males, 51 females, mean age 30.4 yrs, range 1-88 yrs), diagnosed with idiopathic pancreatitis. Genomic DNA was extracted from whole blood samples, amplified by PCR and sequenced using the Sanger method. Multiplex ligation-dependent probe amplification (MLPA) was performed to assess copy number variations (CNVs) using an MLPA kit. Students t-test (unpaired),  $\chi^2$  test or Fisher's exact test was used for analyzing data as appropriate.

**RESULTS:** We identified three types of PRSS1 mutations in 11 patients, including N291 (n=1), R122H (n=1), and G208A (n=9). 16 patients exhibited heterozygous mutations in SPINK1, including c.194+2T>C (n=12) and N34S (n=3). A novel splicing mutation in the SPINK1 gene, c.194+1G>A, was found in a 41 yr old man diagnosed with pancreatic duct stone and chronic pancreatitis. A heterozygous CFTR Q1352H mutation was detected in 8 patients. One patient had a heterozygous CTRC P249L mutation that has been reported to be a high risk variant for pancreatitis. PRSS1 and SPINK1 gene copy numbers were normal in all patients. Weight loss occurred more frequently in patients carrying the G208A mutation, while pancreatic duct stones occurred more frequently in patients with the c.194+2T>C mutation.

**CONCLUSION:** Mutations in PRSS1, SPINK1, and CFTR were associated with idiopathic pancreatitis in contrast to mutations in CTRC. CNVs of PRSS1 and SPINK1 were not detected.

#### E-P03.33

#### A novel compound heterozygous mutation in the URAT1/SLC22A12 in a Japanese patient with renal hypouricemia

K. Ichida<sup>1,2</sup>;

<sup>1</sup>Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan, <sup>2</sup>Division of Kidney and Hypertension, Jikei University School of Medicine, Tokyo, Japan.

Renal hypouricemia is an inherited and heterogeneous disorder characterized by impaired tubular uric acid transport. Exercise-induced acute renal failure and urolithiasis are frequent complications of renal hypouricemia. Urate transporter 1 gene (URAT1/SLC22A12) and glucose transporter 9 gene (GLUT9/SLC2A9) are causative genes for renal hypouricemia (MIM: 220150, 612076). URAT1/SLC22A12 is the main transporter for uric acid reabsorption at the apical membrane of the renal tubules, while GLUT9/SLC2A9 at the basolateral membrane. In Japanese, over ninety percentages of renal hypouricemic patients have URAT1/SLC22A12 mutations and c.774G>A (p.W258X) accounts for about eighty percentages of URAT1/SLC22A12 mutation. We have investigated a middle-aged male patient with renal hypouricemia who had frequent exercise-induced acute renal failures by moderate level exercise. A compound heterozygous mutation in the URAT1/SLC22A12 was found, a c.774G>A and a novel mutation c.935\_997delinstGG. Further consideration is required, as the mutations cannot account for the frequent exercise-induced acute renal failures.

#### E-P03.34

#### The association between the PTPN22 1858C>T variant and T1D Saudi children.

I. Alabdulkareem<sup>1</sup>, I. Altawayjri<sup>2</sup>, A. Alrumaih<sup>2</sup>, A. Al-Mohrej<sup>2</sup>, M. Almalki<sup>2</sup>, S. Alghamdi<sup>1</sup>, A. Nasr<sup>2</sup>, M. AlDubayee<sup>2</sup>;

<sup>1</sup>King Abdullah International Medical Research Center, Riyadh, Saudi Arabia, <sup>2</sup>King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia, <sup>3</sup>King Abdullah Specialized Children's Hospital, King Abdulaziz Medical City, Riyadh, Saudi Arabia.

Type 1 diabetes (T1D) is a chronic disease in which the pancreas cannot produce insulin because of the destruction of  $\beta$ -cells mostly mediated by cytotoxic CD8+ T-cells with help from CD4+ T-cells Protein tyrosine. Recently, the phosphatase non-receptor type 22 (PTPN22) has been reported to be one of the major genes that may involve in the incidence of T1D in children. A single nucleotide polymorphism (SNP) at nucleotide 1858 in codon 620 (C>T; Arg620Trp; rs2476601) in PTPN22 has been reported to be associated with T1DM in North American studies.

PTPN22 gene maps on chromosome 1p13.3-p13.1, encodes lymphoid protein tyrosine kinase (LYP) acts as negative control of T-cell activation and T-cell development. The other high influence genetic markers reported to have linked with T1D incidence in the children, are the human leucocyte

antigen (HLA-DR/DQ) and insulin gene (INS).

This study is aimed to investigate the presence of the rs2476601 SNP among children diagnosed with T1D at King Abdullah children hospital. A total of 24 patients were subjected to PTPN22 gene sequence for the presence rs2476601 SNP.

Our sequencing data showed that the rs2476601 SNP is present in all of the subjects. This pilot study may show some strong association between the T1D and the presence of the rs2476601 SNP in Saudi population.

Our future plan is to expand the number of patients (aiming 1000 subjects) and do high through put sequencing (gene panels) for PTPN22 and other genes (HLA-DR/DQ and INS).

## E-P04 Skeletal, connective tissue, ectodermal and skin disorders

### E-P04.05

#### Characterization of novel mutations in FLG gene in Mexican patients with ichthyosis vulgaris

*M. Rivera-Vega<sup>1</sup>, E. Alarcon-Perez<sup>1</sup>, F. zuñiga<sup>2</sup>, J. Cazarin-Barrientos<sup>3</sup>, L. Gonzalez-Huerta<sup>1</sup>, M. Martinez-Saucedo<sup>1</sup>, J. Toral-Lopez<sup>4</sup>, S. Cuevas-Covarrubias<sup>5</sup>;*

<sup>1</sup>Genetica, Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Genetica, Hospital Regional de Chiapas, San Cristobal de las Casas, Mexico, <sup>3</sup>Dermatologia, Hospital General de Mexico, Mexico DF, Mexico, <sup>4</sup>Centro Medico Ecatepec, ISSEMYM, Edomex, Mexico,

<sup>5</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.

Ichthyosis vulgaris (IV, OMIM: 146700) is a genodermatosis that represents one of the most frequent single-gene disorder in humans beings. With an incidence of 1 in 250 newborns, IV presents a semi dominant inheritance pattern caused by homozygous, compound heterozygous, or heterozygous mutations in the FLG gene that is located on chromosome 1q21. The loss-of-function mutations in the FLG gene are the cause of IV and sometimes are associated with atopic diseases in 37-50% of the cases. The phenotypic characteristics of IV include palmar hyperlinearity, keratosis pilaris, and a fine scale that is most prominent over the lower abdomen, arms, and legs. The aim of the present study is to describe three novel mutations in the FLG gene in patients with IV. Genomic DNA was analyzed through whole exome sequencing, PCR and DNA sequencing analysis in IV patients, non-affected members of the families and in 100 normal controls. STS protein activity was conducted to completely discard X-linked ichthyosis. We detected three novel mutations (p.P487S, p.S1482Y and p.S3962L) in the FLG gene of the subjects with IV. These results add more diversity of the type of mutations in the FLG gene in IV patients.

### E-P04.06

#### Occurrence of the most frequent type of mutation in FLG gene in Mexican patients with vulgar ichthyosis

*L. Gonzalez-Huerta<sup>1</sup>, F. zuñiga<sup>2</sup>, J. Cazarin-Barrientos<sup>3</sup>, H. Urueta-Cuellar<sup>4</sup>, M. Martinez-Saucedo<sup>1</sup>, S. Cuevas-Covarrubias<sup>5</sup>;*

<sup>1</sup>Genetica, Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Genetica, Hospital Regional de Chiapas, San Cristobal de las Casas, Mexico, <sup>3</sup>Dermatologia, Hospital General de Mexico, Mexico DF, Mexico, <sup>4</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.

Ichthyosis vulgaris (IV, OMIM: 146700), a semidominant condition, is caused by homozygous, compound heterozygous, or heterozygous mutation in the filaggrin gene (FLG; 135940). FLG gene is located on chromosome 1q21, which defects seem to also be associated with atopic diseases. IV is one of the most frequent single-gene disorder in human beings. Its incidence is 1 in 250 based on a survey of 6,051 healthy English school-children. The phenotypic characteristics include palmar hyperlinearity, keratosis pilaris, and a fine scale that is most prominent over the lower abdomen, arms, and legs. In the present study, we determined in 15 Mexican patients the presence of the most frequent mutations reported in IV (p.R501\* and c.2282del4) in the FLG gene. IV diagnosis was performed clinically and molecularly. To discard the X-linked diagnosis, we determined the steroid sulfatase activity in leukocytes, PCR and DNA sequencing of the STS gene. DNA sequencing of the FLG gene were conducted through Sanger method. We found the mutation change p.R501\* in four patients; one of them was a compound heterozygous with the mutation p.R501\*/ p.P487S (a novel mutation). The mutation c.2282del4 was not present. These mutations represent 26.6% of the analysed sample what denotes a different occurrence that those reported in other populations.

### E-P04.08

#### Association of *GSTM1*, *GSTT1* and *GSTP1* gene polymorphisms of Lichen Planus in Turkish population

*S. Artan<sup>1</sup>, I. Bultur<sup>2</sup>, H. Aslan<sup>1</sup>, B. Durak Aras<sup>1</sup>, M. Ozdemir<sup>1</sup>, O. Kutlay<sup>1</sup>, E. Erzurumluoglu<sup>1</sup>, K. Haziyeva<sup>1</sup>, Z. N. Saracoglu<sup>2</sup>, O. Cilingir<sup>1</sup>;*

<sup>1</sup>Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Genetics, Eskisehir, Turkey, <sup>2</sup>Eskisehir Osmangazi University, Faculty of Medicine, Department of Dermatology, Eskisehir, Turkey.

Lichen planus (LP) is an inflammatory and chronic immune-mediated skin disease. The oxidative stress and antioxidant defense mechanisms have been identified in the inflammatory and chronic dermatological diseases. *GSTM1*, *GSTT1* and *GSTP1* are important enzymes to utilize many products of oxidative stress as a substrate. Aim of this study is to determine the effects of these antioxidant genes polymorphisms on the molecular etiology of the LP. The study group consisted of 56 patients with LP and 98 age- and sex-matched healthy unrelated controls. *GSTM1* and *GSTT1* genotypes were determined by multiplex PCR, but *GSTP1* polymorphisms were analyzed by using PCR-RFLP technique. Genotype frequencies of the *GSTM1*, *GSTT1* and *GSTP1* polymorphisms showed significant differences between Lichen Planus patients and control populations as are shown in table. The *GSTP1*- Val/Val genotype, *GSTM1*-null and *GSTT1*-null polymorphisms may be connected with LP. Since the analysis of these polymorphisms in LP is the first data published in the world.

Genotype	Comparison of <i>GSTM1</i> , <i>GSTT1</i> and <i>GSTP1</i> frequencies between patients and controls		P value
	LP (n=56) n(%)	Control (n=98) n(%)	
<i>GSTM1</i>			
Null (-)	5(3,2)	0(0)	<0,01
Present (+)	51(96,8)	98(63,6)	
<i>GSTT1</i>			
Null (-)	13(8,4)	0(0)	<0,001
Present (+)	43(91,6)	98(63,6)	
<i>GSTM1</i> / <i>GSTT1</i>			
+/+	38(24,7)	98(100)	<0,001
ile/ile	2(1,3)	15(9,7)	<0,05
ile/Val	47(30,5)	83(53,9)	>0,5
Val/Val	7(4,5)	0(0)	<0,001

### E-P04.09

#### Report of two novel mutations in *FBN1* gene and one novel mutation in *TGFBR2* gene in five Iranian families suspicious to Marfan and Marfanoid syndrome

*E. Esmailzadeh-Gharehdaghi<sup>1</sup>, S. Yazdanipour<sup>2</sup>, M. Garshasbi<sup>1</sup>;*

<sup>1</sup>Tarbiat modares university, Tehran, Iran, Islamic Republic of, <sup>2</sup>Islamic Azad university dentistry, Tehran, Iran, Islamic Republic of.

Marfan syndrome is an autosomal dominant disorder that affects the body's connective tissues with a relative highly prevalence. Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems. This study was performed to investigate mutation spectrum and responsible gene in a cohort of 4 Iranian families with Marfan syndrome and 1 family with possible diagnosis of Marfan syndrome and Aneurysm respectively. All the coding regions of *FBN1* gene in the first four patients were screened by target-enriched next generation sequencing. One patient from the remaining family was subjected to NGS panel for total 14 genes (ACTA2, CBS, *FBN1*, *FBN2*, MYH11, COL3A1, SMAD3, *TGFBR1*, *TGFBR2*, MYLK, MSTN, COL5A2, *TGFB2*, SLC2A10). This study revealed two novel mutations c.6288C>A (p.Cys2096Ter) and c.6793T>G (p.Cys2265Gly) in *FBN1* gene in two of the families and one novel mutation c.1333G>A (p.Gly445Arg) in *TGFBR2* gene which is related to autosomal dominant Loeys-Dietz syndrome in the family with Marfan syndrome and Aneurysm. The confirmation study by sanger sequencing showed that all of these three mutations co segregate with the disease in the three families. In two families no mutation in *FBN1* gene was observed. This result indicates the allele heterogeneity of *FBN1* and *TGFBR2* genes by introducing three novel mutations in five Iranian families.

### E-P04.10

#### Frequency of deletions in the *NF1* gene analyzed through MLPA in a sample of Mexican patients with neurofibromatosis 1

*J. Valdes-Miranda<sup>1</sup>, L. M. Gonzalez-Huerta<sup>1</sup>, J. Cazarin-Barrientos<sup>2</sup>, S. Cuevas-Covarrubias<sup>3</sup>;*

<sup>1</sup>Genetica, Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Dermatologia, Hospital General de Mexico, Mexico DF, Mexico, <sup>3</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.

Neurofibromatosis type I (NF1) (OMIM #162200) presents an occurrence of 1 in 2500-3500 newborns. Clinically, NF1 is characterized by cutaneous café-au-lait spots, skin-fold freckling, Lisch nodules in eyes and neurofibromas. NF1 has an autosomal dominant inheritance with complete penetrance age-dependent. NF1 is caused by molecular defects in the *NF1* gene located

on chromosome 17q11.2, a gene with 60 exons, alternative splicing and expression in most tissues. NF1 encodes neurofibromin 1, a tumor suppressor protein that participates in the regulation of cell growth and proliferation. A large number of mutations are observed in NF1 patients (point mutations, indels and complete gene deletions including flanking regions). The aim of the present study was to determine the frequency of deletions in the NF1 gene through MLPA in a sample of 38 Mexican patients with NF1. MLPA technique was performed in genomic DNA in all patients. Four deletions that include a complete deletion of the NF1 gene and three intragenic deletions were observed. We detected a frequency of gene deletions in the NF1 gene in Mexican population similar to those reported for other populations (5-10%). We observed a higher prevalence of intragenic deletions than complete deletions of the NF1 gene. This type of studies is necessary in most populations to identify the frequency and type of this molecular defect in NF1.

#### E-P04.11

##### NF type 1: a single center's experience in the Aegean region of Turkey

A. Ece Solmaz<sup>1</sup>, H. Onay<sup>1</sup>, E. Isik<sup>2</sup>, T. Atik<sup>2</sup>, F. Ozkinay<sup>1</sup>;

<sup>1</sup>Ege University Medical Faculty Medical Genetics Department, Izmir, Turkey, <sup>2</sup>Ege University Medical Faculty Pediatric Genetics Department, Izmir, Turkey.

**Introduction:** Neurofibromatosis 1 (NF1) is an autosomal dominant condition caused by mutations in the NF1 gene. It is also a multisystem disease that shows high penetrance with a wide variability in the phenotype. The most common features are multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules.

**Materials and Methods:** A total of 8 unrelated Turkish NF1 patients, 2 females and 6 males, were included in this study. All patients met the diagnostic criteria proposed by the NIH. We screened the whole coding and splice site regions of the NF1 gene using next generation sequencing.

**Results:** We identified 8 different NF1 mutations, including 1 missense, 3 nonsense and 4 small deletion mutations. Among these pathogenic variants 5 were novel.

Age of the patients at the time of NF1 molecular diagnosis was 18.4 years. Six patients (75%) had not a family history of NF1. Among 8 patients, Café-au-lait spots were shown in 8 (100%), neurofibroma in 6 (75%), freckling in 4 (50%), and Lisch nodules in 3 (37.5%).

**Conclusion:** It has been shown that NF1 is an extremely heterogenous disease at both clinical and molecular levels in Turkish population as it is in the other populations. Mutation distribution indicate no mutational hot spots within the NF1 gene in our population. This is the first study from Western Turkey investigating mutation spectrum of the NF1 gene.

#### E-P04.12

##### Report of novel mutation of COL1A1 gene in Osteogenesis imperfecta

M. Ebrahimi Nasab, S. Asadollahi, M. Seyedhassani;

Dr. Seyedhassani Medical Genetic Center, Yazd, Iran, Islamic Republic of

**Introduction:** Osteogenesis Imperfecta (OI) is a heritable disorder characterized by increased bone fragility. Previous studies suggested that the majority of the inheritance of familial OI may be autosomal dominant. *COL1A1/2* genes related OI are characterized by fractures with minimal or absent trauma, variable dentinogenesis imperfecta, and in adult years, hearing loss. The clinical features of *COL1A1/2*-related OI represent a continuum ranging from perinatal lethality to individuals with severe skeletal deformities, mobility impairments, and very short stature to nearly asymptomatic individuals with a mild predisposition to fractures, normal dentition, normal stature, and normal life span. Fractures can occur in any bone, but are most common in the extremities. The Aim of this study was to molecular assessment of the mother and daughter of family with OI.

**Materials and Methods:** We have evaluated mother (Proband) with panel DX0195 by Next Generation Sequencing (NGS) test that included 13 genes related to OI. NGS detected a mutation (c.2613+6 T>C) that occurred in intron 37 of *COL1A1* gene. Her husband and daughter were assessed for this mutation by PCR-Sequencing method.

**Result:** Our data shown her daughter had the same mutation and is very likely suffering from osteogenesis imperfecta.

**Conclusion:** Novel variant c.2613+6T>C (Het) on gene *COL1A1* has not been reported for its pathogenicity. So according to our study this mutation can be considered as a pathogenic mutation. Bioinformatics analysis confirmed this pathogenicity.

#### E-P04.17

##### Novel RECQL4 mutation causing Rothmund-Thomson syndrome

D. Halperin<sup>1</sup>, M. Drabkin<sup>1</sup>, Y. Yoge<sup>1</sup>, Y. Perez<sup>1</sup>, O. Birk<sup>1,2</sup>;

<sup>1</sup>The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer sheva, Israel, <sup>2</sup>Genetics Institute, Soroka University Medical Center, Ben-Gurion University, Beer sheva, Israel.

Three siblings of a consanguineous Bedouin Israeli kindred presented with a syndrome of premature aging with poikiloderma and skin atrophy, thin hair and unique dysmorphism – with frontal bossing, small sunken eyes, saddle nose and a small mouth. Severe intrauterine growth retardation (IUGR) was evident in all cases, culminating in severe failure to thrive (FTT). Osteoporosis – leading to multiple fractures, was evident in all 3 affected individuals and absence of thumbs was evident in one. Two of the affected individuals died by the age of 2 years.

Whole exome sequencing of the affected individuals identified 8 homozygous mutations shared by all 3. Filtering of the whole exome sequencing data for known benign variants using open access databases (HapMap, EVS and 1000 genomes) and our in-house data of over 100 ethnically matched exomes, identified a single deleterious homozygous mutation relevant to the phenotype: c.1038\_1039delCC in *RECQL4* resulting in a frameshift and premature stop codon of the mature protein: p.R347fs\*2. *RECQL4* mutations have been previously associated with several syndromes, namely: Rothmund-Thomson (omim#268400), Baller-Gerold (OMIM#218600) and Rapadilino (OMIM#266280). *RECQL4* encodes a DNA helicase protein and the novel mutation identified in this study is of the few described to date that cause Rothmund-Thomson syndrome. The molecular developmental mechanisms through which different syndromes evolve due to mutations affecting this protein are yet to be elucidated.

#### E-P04.18

##### Association between transforming growth factor beta 1 gene and idiopathic scoliosis

S. Nikolova<sup>1</sup>, V. Yablanski<sup>2</sup>, E. Vlaev<sup>2</sup>, A. Savov<sup>1</sup>, I. Kremensky<sup>3</sup>;

<sup>1</sup>National Genetic Laboratory, Medical University-Sofia, Sofia, Bulgaria, <sup>2</sup>Department of Orthopedics and Traumatology, Tokuda Hospital Sofia, Sofia, Bulgaria, <sup>3</sup>Molecular Medicine Center, Medical University-Sofia, Sofia, Bulgaria.

**Introduction.** The transforming growth factor-beta (TGF- $\beta$ 1) functional polymorphism rs1800469 (C-509T) corresponding with a higher transcriptional activity than the wild-type variant and with higher levels of the gene product was previously described to be associated with the etiology and progression of idiopathic scoliosis in Russian population. The purpose of our case-control study was to investigate the association between TGF- $\beta$ 1 (rs1800469 C/T) and idiopathic scoliosis in Bulgarian population.

**Materials and Methods.** The association study was performed on 105 patients and 210 controls. The mean Cobb angle was  $54.6^\circ \pm 22.70$  and the mean age of the patients was  $11.2 \pm 3.1$  years. After obtaining written informed consent peripheral blood samples were collected and genomic DNA was extracted automatically. The genotyping was carried out by TaqMan Real-Time PCR method. The statistical analysis was performed by Pearson Chi-squared test with p-value less than 0.05 as statistically significant.

**Results.** The frequencies of the polymorphic T allele and TT genotype in the total group of patients and in the subgroup of patients with Cobb angle above  $40^\circ$  were significantly higher than that in the controls ( $p < 0.05$ ). In addition, this case-control study revealed statistically significant association between TGF- $\beta$ 1 (rs1800469) and idiopathic scoliosis in females, early and late onset idiopathic scoliosis, familial and sporadic forms.

**Conclusions.** The results confirmed a previously reported association between the TGF- $\beta$ 1 gene and idiopathic scoliosis in Russian population and suggested that the molecular marker TGF- $\beta$ 1 (rs1800469 C/T) is independent predisposing and modifying factor of idiopathic scoliosis in Bulgarian patients.

#### E-P05 Cardiovascular disorders

##### E-P05.01

##### Polymorphisms in autophagy genes are associated to Acute Coronary Syndrome

R. Usategui-Martin<sup>1</sup>, A. Medina Herrera<sup>1</sup>, B. Crego-Vicente<sup>1</sup>, O. Cortes-Fernández<sup>1</sup>, E. Bueno-Martínez<sup>1</sup>, I. Cruz-González<sup>2</sup>, J. Rama-Merchán<sup>2</sup>, P. Sánchez-Fernández<sup>2</sup>, R. González-Sarmiento<sup>1</sup>;

<sup>1</sup>Molecular Medicine Unit. Department of Medicine. University of Salamanca- Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain, <sup>2</sup>Department of Cardiology. University Hospital of Salamanca- Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.

Acute coronary syndrome (ACS) is the main cause of death in the European Union. ACS includes a series of acute myocardial ischemic states as unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). ACS is the result of the formation of occlusive thrombi (STEMI) or no occlusive thrombi (NSTEMI) in the coronary arteries resulting to rupture of vulnerable atherosclerotic plaques. As a result of energy depletion due to the ischemic process an activation of the autophagy occurs to replenish metabolic substrates and to remove damaged organelles. So the aim of our study was to characterize whether polymorphisms in genes involved in autophagy would modify the risk of developing ACS. We have studied ATG2B rs3759601, ATG16L1 rs2241880, ATG10 rs1864183 and ATG5 rs2245214 polymorphisms to evaluate their role in the susceptibility of suffering ACS in a cohort of 323 Spanish patients.

Our results showed that being a carrier of the allele G of ATG2B rs3759601 polymorphism and being a carrier of the allele C of the ATG16L1 rs2241880 polymorphism were associated with increased risk of developing ACS. The analysis of the NSTEMI patients versus STEMI patients showed us that being a carrier of the variant allele G of the ATG2B rs3759601 polymorphism increased the risk of developing STEMI.

The variant alleles of the ATG16L1 rs2241880 and the ATG2B rs3759601 polymorphisms are related with decreased autophagy process therefore our study suggests that a defective autophagy could be involved in an increased risk to suffer ACS.

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#### E-P05.09

##### Paternal lineage I as a risk factor of coronary artery disease in Slovak men

E. Petrejčíková<sup>1</sup>, M. Fecenková<sup>1</sup>, J. Hubcejová<sup>1</sup>, I. Boroňová<sup>1</sup>, I. Klobušovská<sup>1</sup>, J. Kmec<sup>2</sup>;

<sup>1</sup>Prešov University, Faculty of Humanities and Natural Sciences, Department of Biology, Prešov, Slovakia, <sup>2</sup>Kardiocentrum, FNsP J.A.Reimana, Prešov, Slovakia.

**Introduction:** Coronary artery disease (CAD) is a major cause of high mortality in Slovakia. Generally, men have a higher risk than women of developing coronary heart disease in middle age. We assumed that genetic variability in the Y chromosome (especially haplogroup I) could be a risk factor for a higher incidence of coronary artery disease in the Slovak male population independently of traditional cardiovascular risk factors.

**Materials and Methods:** Genomic DNA was extracted from peripheral blood and buccal swabs of 133 Slovak patients with CAD and 127 control samples. Males were genotyped for Y-SNPs on 7500 Fast Real Time PCR instrument (Life Technologies) using TaqMan assays. We compared traditional cardiovascular risk factors in carriers of the haplogroup I and in men with other paternal lineages.

**Results:** Based on analysis, haplogroup I was no significantly more common in men with coronary artery disease than in controls (24.81% [n=33] vs. 25.2% [n=32]). There were no significant differences in biochemical and clinical parameters between patients with paternal lineages I and patients with other Y-haplogroup. We did not confirm the association between haplogroup I and increased risk of coronary artery disease in this study (OR 0.9797, 95% CI 0.5578 to 1.7177, p=0.942).

**Conclusion:** In Slovak population, our results showed the carriers of haplogroup I are not more likely to suffer from coronary artery disease than men with other paternal lineage. None of the traditional cardiovascular risk factors associated with Y-chromosomal haplogroup I.

This study is the result of the project implementation VEGA 1/0563/14.

#### E-P05.10

##### Next-generation sequencing to determine a genetic cause of familial intracranial aneurysms

E. Hitchcock<sup>1,2</sup>, J. Diamond<sup>2</sup>, P. Eydoux<sup>1,3</sup>, K. Townsend<sup>1,2</sup>, G. Redekop<sup>1,4</sup>, W. Gibson<sup>1,2</sup>;

<sup>1</sup>University of British Columbia, Vancouver, BC, Canada, <sup>2</sup>Child and Family Research Institute, Vancouver, BC, Canada, <sup>3</sup>Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada, <sup>4</sup>Vancouver General Hospital, Vancouver, BC, Canada.

**Introduction:** Intracranial berry aneurysms (IBA) are a common disease that occur in 1-3% of the general population, and in 3.6-6.5% of adults over 30 years of age. Approximately 12-15% of patients have affected first-degree relatives, and are considered to have familial intracranial aneurysms (FIA). No specific genetic variants causing isolated IBA or FIA have been identified thus far. The aim of this study is to identify rare DNA variants that are major risk factors for FIA.

**Methods and Results:** This study currently has enrolled three families with FIA, and recruitment of additional families is ongoing. We analyzed family one using whole-genome SNP microarrays to map genomic regions shared only among affected family members, in combination with whole-exome sequencing (WES) to identify rare genetic variants in a single proband. Over-

lap of these data produced a list of 112 candidate variants. Whole-genome sequencing will be performed on the proband of family two. WES will be performed on family three and on the probands of newly-enrolled families. We describe a detailed workflow of the analysis of sequencing data obtained from these families. Candidate variants were selected for being rare and potentially damaging, then subsequently prioritized by the gene's involvement in an associated syndrome, vascular function, or inflammation. Genes containing candidate variants in multiple independent families will be modeled in zebrafish.

**Discussion:** A validated FIA gene would be expected to identify a key protein in the molecular pathogenesis of intracranial aneurysms in both its familial and sporadic forms.

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#### E-P05.11

##### Hereditary haemorrhagic telangiectasia (Rendu-Osler-Weber syndrome): molecular genetic testing in the Czech Republic- update

J. Něcasová<sup>1</sup>, Z. Svestková<sup>1</sup>, J. Nemecková<sup>1</sup>, M. Penka<sup>2,3</sup>, R. Gaillyová<sup>1,3</sup>, I. Valasková<sup>1,3</sup>;

<sup>1</sup>The University Hospital Brno, Department of Medical Genetics, Brno, Czech Republic,

<sup>2</sup>The University Hospital Brno, Department of Clinical Hematology, Brno, Czech Republic,

<sup>3</sup>Masaryk University, Faculty of Medicine, Brno, Czech Republic.

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular disorder caused by mutations in endoglin (ENG) or activin receptor-like kinase-1 (ALK1, ACVRL1) genes. Clinical diagnosis is based on the presence of two (suspected) or more (definite) Curaçao's diagnostic criteria. The majority of mutations reported on the International HHT Mutation Database (hhtmutation.org) are predicted to lead to stop codons, either due to frameshifts or direct nonsense substitutions. We reported our first results with genetic testing in the Czech Republic previously. We used classical sequencing approaches to perform molecular characterization in 12 new clinically affected individuals. Coding regions and exon/intron boundaries of both genes were sequenced and we detected a total of 8 different mutations in the two genes. Three known missense mutations were identified in the ACVRL1 gene, in exons 7 and 8. Three of five mutations identified in the ENG gene were novel with frameshift effect in exons 5 and 7, and two were known mutations- one missense in exon 5 and splice site mutation in intron 8. No mutations were found in ENG/ACVRL1 in 4 probands. Our laboratory has performed genetics testing for HHT on 28 probands since 2012. Approximately 54% of affected patients have at least one mutation in either endoglin or ACVRL1 gene. We plan to perform deletion/duplication analysis using MLPA (Multiplex ligation-dependent probe amplification analysis) in all patients with HHT retrospectively.

#### E-P05.12

##### Genetics Contribution in Blood pressure and Hypertension

S. Zoroufchin Tamiz, M. S. Fallah, M. S. Daneshpour;

Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

**Introduction:** Hypertension is an emerging public health problem and one of the most frequent risk factor for cardiovascular disease. Genetic of hypertension varies from monogenic to polygenic-multifactorial forms which the latter is developed by interaction of various genes and environmental factors. Here we present a narrative review to further elucidating the genetics of elevated blood pressure (BP).

**Material and Methods:** Literature was searched through PubMed, dbGap and HuGE navigator and Google Scholar using a combination of following terms: hypertension or BP, genetic, genome wide association study (GWAS). Those manuscripts having data regarding the genes involving in monogenic hypertension or essential hypertension was included. After shortlisting related papers, most significant related genes and SNPs with monogenic and polygenic hypertension was included including most relevant studies such as WTCCC, CHARGE study, BRIGHT study, ICBP-GWAS, The Global BPgen consortium.

**Results:** BP heritability is estimated to be 30% to 50%, but GWAS results failed to unravel it. Large amount of this proposed heritability remained to be discovered which could be explained by primary overestimation of this measure. Limitation of GWAS to uncover underlying genetic cause might be due to not covering all common SNPs and the role of rare variants and lack of GWAS efficiency to detect them.

**Conclusions:** Polygenic characteristic of BP which is manifested by smaller effect of large number of variant needs further studied and the result could alter the focus of disease management from treatment toward prevention, also personalized therapy by using different drug to remedy individuals with specific genotypes.

## E-P05.14

**The development of a novel hypoxia inducible system to treat cardiovascular diseases caused by ischemia**

**M. Gunduz, S. Cigdem, A. Celik, T. Kaya, B. Eryonucu, M. Acar, E. Gunduz;**  
Turgut Ozal University, Ankara, Turkey.

Tissue hypoxia, or ischemia, is the condition that describes the poor conveyance of oxygen and other vital products to tissues and organs. Up to now, the number one cause of death worldwide is caused by ischemia and related conditions such as heart attack or stroke. HIF-1 $\alpha$  is a transcription activator that functions as a master regulator of oxygen homeostasis. HIF-1 $\alpha$  protein levels increase under hypoxic conditions as a result of decreased O<sub>2</sub>-dependent prolyl-hydroxylation, ubiquitination and degradation.

We aimed to break up clots in blood vessels and to prevent damage caused by reperfusion by producing antioxidant enzymes using hypoxia inducible systems. We added oxygen dependent degradation (ODD) domain of HIF1 $\alpha$  between and in front of TetR DNA binding domain and VP16 transactivation domain, so that TetR-ODD-VP16 or ODD-TetR-VP16 could activate transcription of therapeutic genes controlled by tetracycline response element (TRE), in a HIF1 $\alpha$  independent manner. In addition, we also designed therapeutic genes under control of hypoxia response element (HRE) of HIF1 $\alpha$  target genes. Western blotting and immunofluorescence assay results showed the expression and nuclear localization of TetR-ODD-VP16 and ODD-TetR-VP16 constructs under hypoxic conditions, but not normoxic. In addition, using fluorometric reporter systems we proved functionality of these constructs under hypoxic conditions.

In conclusion, we developed hypoxia responsible systems that can be engineered into endothelial cells to prevent ischemia related cardiovascular diseases.

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## E-P05.15

**Jervell-Lange Nielsen Syndrome: you can (not) miss it!**

**S. J. R. Joosten<sup>1</sup>, M. Voorendt<sup>1</sup>, S. Salemink<sup>1</sup>, R. B. Tanke<sup>1</sup>, A. van den Wijngaard<sup>2</sup>, C. L. M. Marcelis<sup>1</sup>;**

<sup>1</sup>Radboudumc, Nijmegen, Netherlands, <sup>2</sup>Academic Medical Center Maastricht, Maastricht, Netherlands.

**Introduction** ~ Jervell-Lange Nielsen Syndrome (JLNS) is an autosomal recessive variant of familial long QT syndrome (LQTS). JLNS is caused by homozygous or compound heterozygous mutations in the KCNQ1- or KCNE1-gene. JLNS is characterized by congenital profound bilateral hearing loss, a long QT interval and ventricular tachyarrhythmias.

**Case** ~ The index patient presented with a cardiac arrest at the age of 15 years. The electrocardiogram (ECG) showed typical signs of LQTS. The boy was already known by a paediatric neurologist, because of delayed development with a seizure disorder (normal EEG) and an ENT specialist, because of congenital deafness.

Two of his brothers were also diagnosed with deafness at birth and seizures at young age. One of them died suddenly at the age of 4. ECG tracing of the other brother showed also signs of LQTS.

Genetic testing of both the index patient and his brother showed a compound heterozygous mutation (c.644dupT and c.1343dupC) in the KCNQ1-gene, which confirm the diagnosis of JLNS.

**Conclusion** ~ The index patient and his brother were deaf since birth and diagnosed at a young age with seizures. One brother died suddenly at an age of 4 and was also deaf since birth. This family was diagnosed with JLNS just after the cardiac arrest of the index patient. The diagnosis JLNS could have been recognised much earlier in this family, because of the typical combination of deafness, recurrent collapses and sudden death in this family.

## E-P05.16

**A novel mutation in KCNQ1 gene in a Moroccan family with Jervell and Lange-Nielsen syndrome**

**N. Adadi<sup>1,2</sup>, N. Lahrouch<sup>3</sup>, I. Ratbi<sup>1</sup>, R. Bouhouch<sup>4</sup>, I. Fellat<sup>4</sup>, R. Amri<sup>4</sup>, C. Bezzina<sup>3</sup>, A. Sefiani<sup>1,4</sup>;**

<sup>1</sup>Centre de génomique humaine, Faculté de médecine et pharmacie, Mohammed V University in Rabat, Morocco, Rabat, Morocco, <sup>2</sup>Institut National d'hygiène, Rabat, Morocco, <sup>3</sup>Department of Experimental Cardiology, Meibergdreef 9, Amsterdam, The Netherlands, Amsterdam, Netherlands, <sup>4</sup>Electrophysiologie et Stimulation cardiaque, Clinique Belvédère, Rabat Morocco, Rabat, Morocco.

Jervell and Lange-Nielsen syndrome (JLNS; MIM 220400) is a rare autosomal recessive cardioauditory ion channel disorder that affects 1/200000 to 1/1000000 children. It is characterized by congenital profound bilateral sensorineural hearing loss (SNHL), a long QT interval, ventricular tachyarrhythmias, and episodes of torsade de pointes on the electrocardiogram. Cardiac symptoms arise mostly in the early childhood and consist of syncopal episodes during periods of stress, exercise, or fright and are associated

with a high risk of sudden cardiac death. JLNS is caused by homozygous or compound heterozygous mutations in KCNQ1 on 11p15.5 or KCNE1 on 1q22.1-q22.2. We report on a 10 year-old boy with congenital hearing loss, a severely prolonged QT interval who presented with multiple episodes of syncope. His parents are first-degree cousins. We performed Sanger sequencing and identified a novel homozygous variant in KCNQ1 (c.1343dupC, p.Glu449Argfs\*14). Both parents were heterozygous carriers of this mutation. The identification of the genetic substrate in this patient confirmed the clinical diagnosis of JLNS and allowed us to provide appropriate management to the patient and genetic counseling to his family. In addition, this finding contributes to our understanding of genetic disease in the Moroccan population.

## E-P05.17

**The impact of Prothrombin G20210A mutation on young-onset stroke in Lithuanian population**

**V. Tatarunas<sup>1</sup>, R. Vainorienė<sup>1</sup>, D. Cereskevičius<sup>1</sup>, I. Valanciūtė<sup>1</sup>, V. Asmonienė<sup>1</sup>, V. Lesauskaite<sup>2</sup>;**

<sup>1</sup>The Hospital of Lithuanian University of Health Sciences Kauno klinikos, Kaunas, Lithuania, <sup>2</sup>The Institute of Cardiology of Lithuanian University of Health Sciences, Kaunas, Lithuania.

**Introduction:** The aim of this study was to analyze carriers of the Prothrombin G20210A mutation having thrombotic complications, to identify patients with young-onset stroke and to find the distribution of this mutation in a random sample of the population.

**Materials and methods:** four cases of patients, who had been hospitalized due to thrombotic complications at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos from 2014 until 2016, were analyzed. A random sample of 475 subjects was studied in the laboratory of Molecular Cardiology of the Institute of Cardiology of Lithuanian University of Health Sciences by using commercial Taqman probes (Applied Biosystems, UK).

**Results:** The 4 patients were carriers of a G20210A heterozygous G/A genotype. None of them carried the FV:Leiden p.R506Q mutation. Three of the represented patients had thromboembolic complications. These three patients were males aged 20.4, 36.4 and 45.3 years. One female patient aged 1.4 years had an ischemic stroke. Thus, one of four patients with a prothrombotic state and a G/A genotype had an ischemic stroke. Population analysis revealed that only (n=4) 0.8 % of G/A prothrombin G20210A heterozygotes and (n=1) 0.2 % of A/A homozygotes were present in a random sample of Lithuania's population.

**Conclusions:** Our results show that the Prothrombin G20210A is a very rare mutation in the Lithuanian population. However, results of this study also show that the Prothrombin G20210A mutation might significantly increase the risk of young-onset stroke.

**E-P06 Metabolic and mitochondrial disorders**

## E-P06.01

**BCAP31 associated global developmental delay, sensorineural hearing loss and movement disorder mimicking mitochondrial encephalopathy in a male**

**S. Albanyan, A. Altenajai, N. Monfared, S. Mahmutoglu;**  
The Hospital for Sick Children, Toronto, ON, Canada.

**Introduction:** BAP31, encoded by BCAP31 located on Xq28, is involved in the export of transmembrane proteins from the endoplasmic reticulum. Pathogenic variants in this gene have been reported in patients with global developmental delay (GDD), dystonia, deafness and dysmorphic features. We report a new patient with BCAP31 associated GDD, bilateral sensorineural hearing loss, generalized dystonia and choreoathetosis. Case Report

**and Results:** This 3.5-year-old boy presented with microcephaly and failure to thrive within the first 3 months of life. His brain MRI showed bilateral increased signal intensity in globus pallidus at age 3 months raising the suspicion of mitochondrial encephalopathy. His muscle biopsy showed pleomorphic subsarcolemmal collection of mitochondria in electron microscopy. His respiratory chain enzyme activities were normal. Various targeted next generation sequencing panels for mitochondrial disorders were negative. He was enrolled to a whole genome sequencing research study (Genome Clinic, Center for Genomic Medicine, The Centre for Applied Genomics) for mitochondrial disorders. He had a hemizygous pathogenic variant, c.533\_536dup (p.Ser180AlafsX6), in BCAP31. His family history was positive for sensorineural hearing loss in his mother who had normal cognitive functions. She was heterozygous for same variant. Conclusions: We report

a new patient with *BCAP31* associated GDD, microcephaly, failure to thrive, dystonia and choreoathetosis. We also report for the first time a symptomatic heterozygous mother. Clinical features, muscle histopathology, brain MRI features as well as family history were suggestive of mitochondrial encephalopathy. Whole genome sequencing confirmed the diagnosis of *BCAP31* in our patient.

#### E-P06.02

##### The association between chronic periodontitis, type 2 diabetes mellitus and polymorphisms in IL6 and VDR gene

C. Cristescu<sup>1</sup>, A. Craciun<sup>2</sup>, S. Spandole-Dinu<sup>3</sup>, I. Radu<sup>3</sup>, A. Alexiu<sup>3</sup>, C. Sinca<sup>3</sup>, M. Toma<sup>3</sup>, C. Serafinceanu<sup>2</sup>, L. Bercat<sup>4</sup>, D. Cimponeriu<sup>2</sup>,

<sup>1</sup>Titu Maiorescu University, Bucharest, Romania, <sup>2</sup>Nicolae Paulescu NIDNMD, Bucharest, Romania, <sup>3</sup>University of Bucharest, Bucharest, Romania, <sup>4</sup>National Institute of Research and Development for Food Bioresources, Bucharest, Romania.

Complex interactions between periodontal pocket microbiota, host immune response and other risk factors (e.g. smoking, metabolic diseases) have been described. Thus, there are potential contribution of vitamin D receptor and IL6 polymorphisms to predisposition for chronic periodontitis (CP) or type 2 diabetes mellitus (T2DM). Torque teno virus (TTV) produces chronic infections and can interfere with IL-6 production and secretion.

Aim. The aim of this study was to evaluate the association between four genetic polymorphisms and CP or T2DM in Romanian population.

Material and methods. Patients with CP without T2DM (n=150), T2DM with CP (D-CP=150) and matched clinically healthy subjects (n=300) were recruited for this study. The rs2228570 C>T, rs7975232 G>T, rs731236 T>C from VDR locus and rs1800795 from IL6 locus were genotyped by PCR based methods polymorphisms. In addition, the presence of Torque Teno viruses in all samples was detected by nested-PCR.

Results. Torque Teno viruses were detected with similar frequency in all groups. The distribution of genotypes in all lots is not different from the Hardy-Weinberg equilibrium. Evidence of association between D-CP were obtained for rs1800795 in combination with TTV (p<0.05). The VDR genotypes or the haplotype are simmilar distributed in patients and control lots. No significant association was detected between investigated polymorphisms and age at diagnosis of CP or T2DM.

Conclusions: We found that association between rs1800795 and TTV can change the risk for chronic periodontitis in diabetic patients from Romania.

#### E-P06.06

##### Fabry Disease: Report of a mexican family with a previously unreported mutation in exón 6 of the GLA gene (c.968C>G p.P323R)

I. P. Dávalos Rodríguez<sup>1</sup>, M. A. Aceves-Aceves<sup>1</sup>, S. A. Alonso-Barragán<sup>1</sup>, I. M. Salazar-Dávalos<sup>1</sup>, L. E. Wong-Ley Madero<sup>2</sup>, C. A. Ramírez-Aréchiga<sup>3</sup>, E. Cervantes-Pérez<sup>4</sup>, J. J. Magallanes-Ordoñez<sup>4,5</sup>, D. García-Cruz<sup>1</sup>, N. O. Dávalos-Rodríguez<sup>1,6,7</sup>;

<sup>1</sup>Instituto de Genética Humana Dr. Enrique Corona, CUCS, Universidad de Guadalajara, Guadalajara, Mexico, <sup>2</sup>Hospital Civil de Tepic, Tepic, Mexico, <sup>3</sup>Departamento de Bienestar y Desarrollo Sustentable, Centro Universitario del Norte, U de G, Colotlán, Mexico, <sup>4</sup>Doctorado en Farmacología, CUCS, Universidad de Guadalajara, Guadalajara, Mexico, <sup>5</sup>Hospital Regional "Dr. Valentín Gómez Farías" Cirugía Plástica, Zapopan, Mexico, <sup>6</sup>Hospital General de Occidente SSJ, Zapopan, Mexico, <sup>7</sup>Hospital Regional "Dr. Valentín Gómez Farías" Genética, ISSSTE, Zapopan, Mexico.

Introduction: Fabry Disease (FD) is a lysosomal storage disorder caused by a deficiency of the enzyme  $\alpha$ -galactosidase A. It is an X-linked trait caused by a mutation in the GLA gene on Xq22.1. The phenotype/genotype in FD is heterogeneous. FD causes glycolipids to accumulate in the vascular and endothelium.

Objective: We present the case of a 3 generation family where five individuals are identified as carriers of a previously unreported heterozygous variant of FD in exón 6 of the GLA gene.

Case report: The proposita is a 9 month-old female, product of non-consanguineous parents. At 24 hours of birth, bilateral subconjunctival hemorrhage and jaundice appear, remaining in intensive care for breathing difficulties, heart murmur and hypertension. Renal ultrasound: ectasia in left kidney, small right renal artery and right renal hypoplasia. FD Testing: Gene Sequencing GLA heterozygous variant in exon 6 of the GLA gene (c.968C>Gp. P323R). HPLC/Tandem MS lyso Gb3 0.8ng/ml (reference<0.8/ml). Family sequencing studies: 4 members (mother, maternal: aunt, cousin and grandfather), with same mutation. Normal Gb3. None of them had F.D. symptomatology.

Discussion: In this case only the proposita presents clinical manifestations of FD at birth. There are no reports of infants with these findings. In this variety the genotype/phenotype association appear to be independent findings.

Conclusions: FD has a high genetic heterogeneity. Molecular studies and

genotype-phenotype correlation are required to clarify the variability. Family clinical and molecular studies are required to prevent complications and implement enzyme replacement therapy to improve the quality of life if necessary.

#### E-P06.07

##### A novel GLA mutation in a Maltese patient with early-onset Fabry disease

I. Borg<sup>1,2</sup>, C. Apap<sup>3</sup>, S. Aquilina<sup>2</sup>, S. Pirotta<sup>4</sup>, G. Sciriha<sup>4</sup>, M. Vella<sup>4</sup>, S. Eichler<sup>5</sup>, A. Rolfs<sup>6,5</sup>,

<sup>1</sup>Department of Pathology, University of Malta, Msida, Malta, <sup>2</sup>Medical Genetics Unit, Mater Dei Hospital, Msida, Malta, <sup>3</sup>Dermatology Department, Sir Paul Boffa Hospital, Floriana, Malta, <sup>4</sup>Ophthalmology Department, Mater Dei Hospital, Msida, Malta,

<sup>5</sup>Centogene AG, Rostock, Germany, <sup>6</sup>Albrecht Kossel Institute for Neuroregeneration, University of Rostock, Medical Faculty, Rostock, Germany.

Fabry disease is an X-linked lysosomal storage disorder. Mutations in the GLA gene result in deficient alpha galactosidase A enzyme activity and subsequent progressive accumulation of globotriaosylceramide (GL-3) in the vascular endothelium and various body organs.

Here we report a novel hemizygous mutation p.Lys240ArgfsX29 in exon 5 of the GLA gene in a 15 year old male who presented with angiokeratomas in the trunk region, pain in the body extremities, gastrointestinal pain and cornea verticillata. Alpha galactosidase A activity was 0.0078mU/mg (normal range 0.36-0.84 mU/mg). This mutation was subsequently found in his asymptomatic 45 year old mother. No other family members were found to carry this mutation. Concentration of the biomarker Lyso-Gb3 was markedly increased at 195ng/ml (normal reference <0.9 ng/ml).

In view of the clinical phenotype and the very low alpha galactosidase A activity we conclude that this novel mutation is the cause of Fabry disease in this patient.

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#### E-P06.08

##### Primary familial hypercholesterolemia (FH) in children might be caused by different genes

I. M. Abramova<sup>1</sup>, E. Hramtsova<sup>1</sup>, A. Y. Vasina<sup>2</sup>, I. Y. Melnikova<sup>1</sup>, V. Larionova<sup>3,1</sup>,

<sup>1</sup>North-Western State Medical University named after I.I. Mechnikov, St. Petersburg, Russian Federation, <sup>2</sup>St. Petersburg State University, St. Petersburg, Russian Federation,

<sup>3</sup>Center For Rare Diseases and Molecular medicine „IEM“ RAS, St. Petersburg, Russian Federation.

The role of the molecular testing is the most important in the early diagnostics of autosomal recessive FH, since lipid level in parents may be normal. Diagnostics of autosomal dominant form of FH (ADH) is possible by both routine laboratory tests and family history evaluation.

Material and Methods: Analysis of mutation in a gene of a receptor of LDLR DeltaG197 exon 4 in two patients with FH. Results: Patient M., 6 years old, with skin xanthomas in palms, elbow and knee joints, inguinal and popliteal folds. The first elements of xanthoma appeared at the age of 4 years old. The patient's lipidogram showed cholesterol 16mmol/l, triglycerides (TG) 4.5 mmol/l, low-density lipoprotein (LDL) 8.52 mmol/l. The family pedigree revealed early heart attacks in relatives of the 2nd and 3rd degree on the maternal line. His mother had tendinous xanthomas at the age of 29 years old and his father has normal lipid analysis. Mutation in LDLR DeltaG197 4 exon was detected in the child, his mother, and the mother's side relatives. Patient A., 14 years old. Lipidogram showed cholesterol-10.8 mmol/l, TG-2.0 mmol/l, HDL-C-1.8 mmol/l, LDL-8.1 mmol/L. Mutation in LDLR gene DeltaG197 exon 4 was not detected. Parents' lipid analyses were normal. Family history was not available.

Conclusions: In the patient M., ADH might be caused by mutation in LDLR gene. In the patient A., normal lipidogram in the parents and relatives did not allow excluding the autosomal recessive FH (ARH1), which can be confirmed by the further analysis of LDLRAP1 gene.

## E-P06.09

## Revealing a novel mutation in GALC gene with Krabbe disease

I. Suer<sup>1</sup>, A. Koparır<sup>1</sup>, R. Celik<sup>2</sup>, A. Onul<sup>2,3</sup>, A. Yüksel<sup>4</sup>, M. Ozen<sup>3,5,6</sup>,<sup>1</sup>Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Turkey, <sup>2</sup>Biruni University, Reyhan Genome Center, Istanbul, Turkey, <sup>3</sup>Biruni University, Department of Medical Genetics/Molecular Biology and Genetics, Istanbul, Turkey, <sup>4</sup>Biruni University, Istanbul, Turkey, <sup>5</sup>Department of Medical Genetics, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey, <sup>6</sup>Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Krabbe disease (KD) is a rare autosomal recessive inherited neurometabolic disorder associated with the mutations in GALC gene. This gene is located in the 14q31 and comprises of 17 exons. 138 mutations have been reported in the GALC gene and the mutations change galactocerebrosidase activity. The clinical findings can be seen in various spectrums depending on galactocerebrosidase activity.

**Material and Method:** In the current study, we present a Turkish male with KD having neurodevelopmental delay, increased spasitis, homocystinuria, seizures, and optic atrophy. Galactocerebrosidase deficiency was demonstrated by biochemical analysis. We utilized next generation sequencing (NGS) as a diagnostic tool to identify the molecular basis of KD and we confirmed suspected variants by Sanger sequencing.

**Result:** We found a novel homozygous c.825C>G p.Asp275Glu missense mutation in exon 7 of GALC and confirmed it by Sanger sequencing. Parents were demonstrated to be heterozygous for this mutation.

**Conclusion:** Finding novel mutations in the related gene of Krabbe disease is beneficial for establishing certain diagnosis and estimating the prognosis of the disease. It is also important for deciding proper treatment alternatives for the physicians.

## E-P06.12

## Case report: Novel IVD gene mutation induces Isovaleric acidemia (IVA)

S. Mohammadi, M. Ebrahimi Nasab, S. Seyedhassani;

Dr. Seyedhassani Medical Genetic Center, Yazd, Iran, Islamic Republic of.

**Introduction:** Isovaleric acidemia (IVA) is an autosomal recessive inborn error of the leucine metabolism that is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD). Isovaleryl-CoA dehydrogenase (IVD) is a mitochondrial matrix enzyme that catalyzes the third step in leucine catabolism. The genetic deficiency of IVD results in an accumulation of isovaleric acid, which is toxic to the central nervous system and leads to isovaleric acidemia. **Materials and Methods:** In this study, the proband with symptoms of IVA referred us. We used the newborn screening for metabolic diseases with GC/MS method that shown an increased significant of C5 organic acid, the result of screening and symptoms of newborn confirmed the Isovaleric acidemia (IVA). Then we evaluated all of the exons and exon-intron boundaries of IVD gene with PCR-Sequencing method.

**Results:** We identified a novel homozygous mutation (c.326 T>C p.L109P) in exon 4 of IVD gene. The heterozygosity of his parents for the mentioned mutation was confirmed by PCR-Sequencing method. There was no report of this mutation in the literature; because this mutation was present in the parents, in heterozygous state, it is very likely to be pathogen. Bioinformatics analysis (with UMD-Predictor, Expacy, UCL-CS, Polyphen and CADD Score) confirmed our studies.

**Conclusion:** Our study showed a novel mutation for IVD. This result can be used for genetic counseling and prenatal diagnosis.

## E-P06.13

## L-2-Hydroxyglutaric aciduria: Identification of a novel mutation in the L2HGDH gene at Turkish Family

H. Bagış<sup>1</sup>, M. Ö. Çevik<sup>1</sup>, I. Güney<sup>1</sup>, B. Ekmekçi<sup>2</sup>, Ö. F. Karaçorlu<sup>1</sup>,<sup>1</sup>University of Adiyaman and Medical Faculty, Department of Medical Genetic, Adiyaman, Turkey, <sup>2</sup>University of Adiyaman and Medical Faculty, Department of Neurology, Adiyaman, Turkey.

**Background:** L-2-Hydroxyglutaric aciduria (L-2-HGA) is a rare inborn error of metabolism caused by mutations in the L-2-hydroxyglutarate dehydrogenase (L2HGDH) gene which is mapped at chromosome 14q22.1, consists of 10 exons. We have identified a novel L2HGDH gene mutation and three L-2-HGA patients at a family.

**Case Presentation:** A 29-year-old man admitted to our clinic with complain of epilepsy and mild intellectual disability. His brain MRI revealed a symmetrical, extensive subcortical white matter lesion and also a cystic lesion at deep white matter. Urine test for organic acids showed a significantly increased level of 2-hydroxyglutaric acid and simultaneous plasma lysine

amino acid level also was very high. These findings suggest us diagnosis of L-2-HGA. After L2HGDH gene sequence analysis, a homozygous variation, c.738G>A (p.Lys246Lys), in exon 6 reported. The variation has no effect on amino acid residue but its location at the last codon of sixth exon and may alter splicing. After further evaluation; his brother and his sister also have similar signs and their molecular analysis showed same c.738G>A change at L2HGDH gene. Parents have third cousin marriage and have no symptoms. Parents' gene analysis confirmed heterozygous single base change at same nucleotide and so diagnosis of siblings' L-2-HGA disease at molecular level.

**Conclusion:** For the understanding associated clinic symptoms and identification of exact mechanisms of this rare disease we have an ongoing project for high consanguinity rates and diseases burden at South-East of Turkey especially at Adiyaman province and at the village of this family. Possible genotype-phenotype correlation will be declared after study.

## E-P06.14

## The Leigh syndrome caused by novel SURF1 mutation detected in Slovakia

D. Danis<sup>1</sup>, M. Skopkova<sup>1</sup>, K. Brennerová<sup>2</sup>, M. Kolníková<sup>3</sup>, T. Kurdičová<sup>1</sup>, B. Ukropcová<sup>1</sup>, J. Ukropc<sup>1</sup>, J. Staník<sup>1,2,4</sup>, I. Klimes<sup>1</sup>, D. Gasperíková<sup>1</sup>;<sup>1</sup>Institute of Experimental Endocrinology, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia, <sup>2</sup>First Department of Pediatrics, Medical Faculty of Comenius University and Children Faculty Hospital, Bratislava, Slovakia, <sup>3</sup>Department of Pediatric Neurology, Medical Faculty of Comenius University and Children Faculty Hospital, Bratislava, Slovakia, <sup>4</sup>Center for Pediatric Research, Hospital for Children & Adolescents, University of Leipzig, Leipzig, Germany.

Leigh syndrome (LS) is a genetically heterogeneous disease caused by insufficient function of the mitochondrial respiratory chain. Typical presentation starts in infancy with psychomotor regression, ataxia, dystonia, lactic acidosis and characteristic MRI findings. Nuclear gene SURF1 encodes an assembly factor for cytochrome c-oxidase complex and is vital for its function. Autosomal recessive mutations in SURF1 cause approximately one third of COX-related LS cases.

**Patients and Methods:** Psychomotor delay, severe hypotonia, hypotrophy, hypoglycemia and elevated blood and CSF lactate were noted in 6 month-old boy. Decreased activities of Complex II and IV were witnessed. Nevertheless, MRI was not pointing to LS at the time. Demyelination of pons, mesencephalon and basal ganglia were observed later at 11 months and rapid progressive deterioration lead to death at the age of 1 year. Blood sample was collected for whole exome sequencing (WES) at the age of 6 months. WES was performed on Complete Genomic's platform. Called variants were loaded into Gemini SQLite database and all nuclear genes encoding mitochondrial proteins were inspected.

**Results:** Two heterozygous deletions c.845\_846del(p.S282Cfs\*9) and c.823\_833+7del(p.I275Vfs\*13) were found in SURF1 (homozygous presence confirmed by allele-specific PCR followed by Sanger sequencing). The c.845\_846del variant is the most common pathogenic variant in SURF1. Previously unreported c.823\_833+7del variant is not present in population databases (dbSNP142, ESP, ExAC, LOVD) and resulting protein would lack functional C-transmembrane domain therefore we consider it pathogenic.

**Conclusions:** We have identified two variants (one novel) in SURF1 which resulted in LS with ambiguous onset but with rapid progression.

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## E-P06.15

## Identification and characterization of the novel point mutation m.3634A&gt;G in the mitochondrial MT-ND1 gene associated with LHON syndrome

L. Carreño Gago<sup>1,2</sup>, J. Gamez<sup>3</sup>, Y. Cámaras<sup>1,2</sup>, E. Alvarez de la Campa<sup>4</sup>, J. Aller-Alvarez<sup>2</sup>, D. Moncho<sup>5</sup>, M. Salvado<sup>3</sup>, A. Galan<sup>6</sup>, X. de la Cruz<sup>4</sup>, T. Pinos<sup>1,2</sup>, E. García-Arum<sup>1,2,7</sup>;<sup>1</sup>Departament de Patología Mitocondrial i Neuromuscular/Hospital Universitari Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain, <sup>2</sup>Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER)/ISCIII, Barcelona, Spain,<sup>3</sup>Departament de Neurología/Hospital Universitari Vall d'Hebron, Barcelona, Spain,<sup>4</sup>Translational Bioinformatics/Hospital Universitari Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain, <sup>5</sup>Departament de Neurofisiología/Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>6</sup>Departament d'Oftalmología/Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>7</sup>Departament of Clinical and Molecular Genetics and Rare Disease Unit/Hospital Universitari Vall d'Hebron, Barcelona, Spain.

Leber's hereditary optic neuropathy (LHON) is a mitochondrial genetic disease characterized by acute or subacute, progressive, and bilateral central visual loss. The most cases of LHON syndrome are caused by point mutations in MT-ND1, MT-ND4 and MT-ND6 genes. We report a homoplasmic novel mutation m.3634A>G in MT-ND1 gene (p.Ser110Gly), in a patient with a classic clinical features of LHON syndrome.

Several observations support the idea that mutation is pathogenic and involved in the clinical phenotype of the patient: 1) the mutation affected a conserved nucleotide and amino acid; 2) in the same amino acid was previously reported a pathogenic mutation (m.3635G>A, p.Ser110Asn) in a patient with LHON syndrome; 3) the mutation was not observed in databases like Mitomap and Human Mitochondrial Genome Database; 4) in silico predictors programs classified the mutation as "probably damaging"; 5) cybrids carrying the mutation presented decreased enzyme activity of complex I and lower cell proliferation and mitochondrial membrane potential respect to control cybrids.

#### E-P06.16

#### Late-onset Tay-Sachs disease (LOTS) with common Ashkenazi mutation in a patient of Russian-Tatar ethnicity

G. E. Rudenskaya, A. M. Bukina, T. M. Bukina, E. Y. Zakharova, D. Pyankov;  
Medical Genetics Research Centre, Moscow, Russian Federation.

**Introduction:** Two variants of GM2 gangliosidosis, Tay-Sachs disease common in Ashkenazi Jews (gene HEXA) and less frequent but occurring globally Sandhoff disease (HEXB), have rare late-onset forms differing from common infantile phenotype and poorly detected clinically.

**Materials and Methods:** We diagnosed LOTS in 27-year-old female of mixed ethnicity (Russian mother and Tatar father). Enzyme and DNA tests were used.

**Results:** The patient coming from Bashkiria (south-east of European Russia adjoining Ural) had typical presentation of adolescence-onset, slow-progressing proximal spinal amyotrophy, spinocerebellar ataxia with cerebellar atrophy on MRI, subclinical polyneuropathy and normal intelligence. We suspected late-onset GM2 gangliosidosis by correspondence 14 years after disease onset. Minding patient's origin, Sandhoff disease seemed probable, but laboratory findings were those of LOTS: decreased hexosaminidase A level with residual activity 4% and HEXA mutation c.805G>A (p.Gly269Ser). Mutation looked like homozygous, but heterozygosity was found only in father and not in mother which may have several explanations: patient's false homozygosity due to maternal HEXA large deletion (though HEXA large deletions are rare), p.Gly269Ser de novo or germinal mosaicism in mother. The origin of mutation in upper generations of family could not be traced. The mutation in homozygous or compound-heterozygous state is common for Ashkenazi LOTS and was also found in few non-Ashkenazi LOTS families, mostly with roots matching regions of historical Ashkenazi settlement (Navon et al, 1990; Neudorfer et al, 2005), but not in Turkic populations as in our case.

**Conclusion:** The case points to wider spread of HEXA p.Gly269Ser mutation than was thought.

#### E-P06.17

#### The face of treated Wolman syndrome

C. Breen<sup>1,2</sup>, S. A. Jones<sup>1,2</sup>, S. Douzgou<sup>1,2</sup>;

<sup>1</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom, <sup>2</sup>Institute of Human Development Faculty of Medical & Human Sciences, University of Manchester, Manchester, United Kingdom.

The natural history of classic early onset lysosomal acid lipase deficiency (also known as Wolman disease) is characterised by hepatosplenomegaly and severe failure to thrive followed by death within 6 months of life. The presence of vacuolated lymphocytes and adrenal calcification may also aid diagnosis although this is confirmed with enzymatic and molecular tests. Treatment with the licensed enzyme replacement therapy sebelipase alfa (Kanuma, Alexion) has led to patients surviving longer than the previously reported life expectancy. As the children have become older, we noted that they share similar craniofacial features. We propose a facial gestalt in early onset lysosomal acid lipase deficiency including down-slanting palpebral fissures, a depressed nasal bridge and mid face hypoplasia.

#### E-P06.19

#### Association of FTO Gene Variants with Metabolic Syndrome (MetS) risk factors in Tehran cardio-metabolic Study (TCGS)

S. Masjoodi<sup>1</sup>, S. Zoroufchin<sup>1</sup>, B. Sedaghatkhayat<sup>1</sup>, M. S. Fallah<sup>1</sup>, K. Guity<sup>1</sup>, F. Azizi<sup>2</sup>, M. S. Daneshpour<sup>1</sup>;

<sup>1</sup>Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid, Tehran, Iran, Islamic Republic of, <sup>2</sup>Endocrine Research Center, Research Institute for endocrine sciences, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran, Tehran, Iran, Islamic Republic of.

**Background:** Metabolic Syndrome (MetS) has many components like waist circumferences and hypertension. Fat mass and obesity-associated gene

(FTO) could play important role in their metabolism. The present study tries to examine the relationship between FTO and CETP gene variants and 2 metabolic syndrome risk factors in affected subjects.

**Materials and Methods:** This association study was conducted in affected MetS 1120 participants in TCGS. Nine different single-nucleotide polymorphisms (SNPs) of FTO and CETP genes (rs1121980, rs1421085, rs1558902, rs8050136, rs7202116, rs6499640, rs1864163, rs3764261, rs1800775) were genotyped using Tetra ARMs-PCR. All data adjusted by age and smoking status. The association of each SNP with MetS parameters was analyzed by Plink software.

**Results:** Four FTO variants (rs1121980, rs8050136, rs1558902 and rs1421085) were associated with waist circumferences and hypertension. Risk-alleles of three first SNPs were significantly associated with diastolic and systolic blood pressure and waist circumferences in the both gender. Strong association were observed in presence of minor allele A at rs8050136 with diastolic and systolic blood pressure and waist circumferences (P dbp = 0.007, p sbp = 0.005, p waist = 0.0008). In rs1421085 the C minor allele showed a significant result males (P dbp = 0.005, p sbp = 0.019, p waist = 0.005).

**Conclusions:** A significant interaction between rs8050136 and diastolic and systolic blood pressure as well as waist circumferences was seen. In conclusion, the presence of A allele could be a predictor of high waist circumferences and increase blood pressure.

#### E-P06.20

#### Implementing the survey of disease natural history as a tool to collect complex information on mitochondrial patients in Lithuania and Latvia

B. Burnyte<sup>1</sup>, I. Inashkina<sup>2</sup>, B. Lace<sup>2</sup>, D. Pelnena<sup>2</sup>, A. Molyte<sup>1</sup>, K. Grigalioniene<sup>1</sup>, V. Kucinskas<sup>1</sup>, A. Utkus<sup>1</sup>;

<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Latvian Biomedical Research and Study Centre, Riga, Latvia.

Mitochondrial disorders (MD) result from impaired cellular energy metabolism and encompass a broad range of conditions with variable manifestations including neuromuscular, cardiac, ophthalmic, auditory, endocrine, and renal involvement. These patients are referred to clinical geneticists by any doctor, mainly pediatrician, neurologist or general practitioner, who suspects a MD diagnosis based on clinical grounds. The objective of this work is to standardize the collection of clinical and research data across Lithuania and Latvia.

Hence, we developed the survey of disease natural history to help us select a group of patients with clinical mitochondrial syndromes and with suspected MD, including respiratory chain deficiency, for a molecular and functional study which is a part of the collaborative project. The survey in use was adapted for both countries in 2015 and includes demographics, genealogy, clinical data, results of biochemical, histochemical and genetic investigations. Nijmegen mitochondrial disease criteria were also added to this survey (Morava E et al, 2006). The tool will serve as a baseline for data to be obtained in 2015-2017. 64 patients were enrolled in 2015. 12.50% of them make up a definite MD group, 73.44% make up a probable MD group and 14.06% are in a possible MD group. Data and biosamples will be accessible to study collaborators and, on request, to other researchers to assist relevant studies. Adopting the survey will improve patient selection and allow us to validate and interpret data from this collaborative project. We will report all preliminary data received during the first year of this study.

#### E-P06.21

#### TYMP Gene IVS9+1G>A mutation is Related with Hypergonadotropic Hypogonadism in Patients with Mitochondrial DNA Depletion Syndrome Type1

B. Çavdarlı<sup>1</sup>, C. Çavdarlı<sup>1</sup>, Ö. Ünal<sup>2</sup>, S. Ceylaner<sup>3</sup>;

<sup>1</sup>Ankara Numune ETH, Ankara, Turkey, <sup>2</sup>Dışkapi Pediatrics ETH, Ankara, Turkey,

<sup>3</sup>Intergene Genetics Center, Ankara, Turkey.

**Introduction:** Mitochondrial DNA depletion syndrome 1 (MIM #603041), also named MNGIE (**Mitochondrial Neurogastrointestinal Encephalomyopathy**) is an autosomally recessive inherited multisystemic disorder caused by mutations in TYMP gene which encodes Thimidine phosphoribosyltransferase, the enzyme of mitochondrial nucleotide synthesis (1). MNGIE is characterised with gastrointestinal dysmotility, peripheral neuropathy, sensorineural hearing loss, ophtalmoplegia, ptosis and mitochondrial energy metabolism defects.

**Material-methods:** A 24 years old male with chronic diarrhea, cachexia and ptosis was evaluated. He was the second child of healthy parents who had third degree consanguineous marriage. Audituar assesment was normal

and external ophthalmoplegia and ptosis were noted by ophthalmologic examination. Myopathic alterations were indicated on electromyography and diffuse patchy intensity increment was seen in subcortical white matter on the cranial MR images. Hormone analyses of the patient revealed elevated FSH and LH levels in relation to his hypoplastic gonads and external genitalia. **Result:** DNA sequence analyses revealed a homozygote IVS9+1G>A mutation in TYMP gene.

**Conclusion:** We report the second patient with hypergonadotropic hypogonadism and a homozygote IVS9+1G>A mutation in TYMP gene (2). Gonadal dysfunction might be the result of mitochondrial defects. MNGIE syndrome patients with a IVS9+1G>A mutation in TYMP gene should be investigated for hypergonadotropic hypogonadism.

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#### E-P06.22

##### Case Report: A novel mutation in Classic Maple Syrup Urine Disease

M. Yavari<sup>1,2</sup>, M. Mirjalili<sup>1</sup>, S. Seyedhassani<sup>1</sup>;

<sup>1</sup>Dr. Seyedhassani Medical Genetic Center, Yazd, Iran, Islamic Republic of, <sup>2</sup>Department of Biology, Faculty of Science, Yazd University, Yazd, Iran, Islamic Republic of.

Introduction: Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disease caused by mutations in the *BCKDHA*, *BCKDHB*, *DBT* and *DLD* genes, which encode the E1 $\alpha$ , E1 $\beta$ , E2 and E3 subunits of the branched-chain  $\alpha$ -keto acid dehydrogenase (BCKD) complex, respectively. This complex is involved in the metabolism of branched-chain amino acids. MSUD can be divided into 4 types, depending on the amount of BCKD enzyme activity present in the affected individuals. The most common type of MSUD is the classic form.

Materials and Methods: In this study, we analyzed the DNA sequences of *BCKDHA* gene in an infant who suffered from MSUD and died at the age of 17 days. Quantitative tandem mass spectrometric analysis was used to assess the levels of branched-chain amino acids in plasma. All of the exons and exon-intron boundaries of *BCKDHA* genes were amplified under standard PCR conditions. PCR products were subjected to Sanger sequencing and output files were aligned to the human genome reference and analyzed by Finch TV 1.4 software.

Results: Screening of all exons and adjacent introns revealed a homozygous deletion (GTTTCA(^47)TCTC<sub>g</sub>GGATGA) as in exon 2 of the *BCKDHA* gene resulting in protein alteration. The heterozygosity of his parents for the mentioned deletion was confirmed by direct sequence analysis of the corresponding segment. This deletion causes a frame shift alteration.

Conclusions: We have identified a novel *BCKDHA* mutation. This finding can be useful in genetic counseling. Prenatal diagnosis is suggested to investigate of the fetus in the next pregnancy.

#### E-P06.25

##### Investigation of The Genetic Factors Which Effect Obesity, Physical Activity Level and Eating Behavior

D. Kirac<sup>1</sup>, T. Avciar<sup>2</sup>, O. Kasimay Cakir<sup>3</sup>, O. Deyneli<sup>4</sup>, D. Yazici<sup>4</sup>, H. Kurtel<sup>3</sup>, E. C. Kaspar<sup>5</sup>, A. I. Guney<sup>2</sup>;

<sup>1</sup>Yeditepe University, Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey, <sup>2</sup>Marmara University, Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkey, <sup>3</sup>Marmara University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey, <sup>4</sup>Marmara University, Faculty of Medicine, Department of Endocrinology and Metabolism, Istanbul, Turkey, <sup>5</sup>Yeditepe University, Faculty of Medicine, Department of Biostatistics, Istanbul, Turkey.

Obesity is a major contributory factor of morbidity and mortality. Besides environment factors, genetic factors may also contribute to the level of physical activity and eating behaviours thus effect obesity. Therefore the aim of this study is to investigate the effect of various gene mutations on obesity, physical activity levels and eating behaviours. 100 patients and 100 healthy individuals were enrolled to the study. Physical activity levels were measured with an accelerometer. Eating behaviours were evaluated using Three-Factor Eating questionnaire. The information about other risk factors were also collected. DNA was isolated from peripheral blood. Mutations of MC4R and the region located 2696 to 3275 bp upstream of the MC4R start codon were investigated with PCR and direct sequencing. 6 mutations in FTO, 1 mutation near MC4R and 1 mutation in NMB were investigated with real-time PCR. Results were evaluated statistically. rs1051168 and rs8050146 mutations were found statistically significant in patients, rs1121980 was found statistically significant in controls. 16 mutations were found in MC4R of

which 14 of them are novel and 8 of them cause amino acid change. 5 mutations were found near MC4R in which 4 of them are novel. Also it was found that, some obesity related factors and questions of TFEQ are associated with various gene mutations. Any relation between gene mutations and physical activity levels were not detected. Due to the genotype data, physical activity demands and eating behaviours, it may be possible to recommend patients for proper exercise and eating patterns to prevent obesity.

#### E-P06.26

##### Isovaleric acidemia : a case report with a new mutation

H. Onder Yilmaz, S. Seyhan, M. Çakir, A. H. Çebi, M. İkbal; Karadeniz Technical University Medicine Faculty, Trabzon, Turkey.

Isovaleric acidemia is a rare, autosomal recessive, isovaleryl coenzyme A dehydrogenase enzyme deficiency disorder associated with an organic acid metabolism. There are two forms of the disease 1-)Acute form (approximately 50% of cases) appear in two weeks of life with lethargy, vomiting and dehydration.2-)Chronic intermittent form occurs after stress or a high protein intake during later childhood. It is seen in 1/250,000 live births. Mutations in the IVD gene causes isovaleric acidemia. Up to now there is 25 defined mutation in IVD gene. In this study, we present a 8-years-old male who was brought to our emergency clinic with vomiting, fever and abdominal pain. The patient was hospitalized with acute pancreatitis. Two years ago he has been determined with same diagnosis. Cystic fibrosis whole gene sequence analysis was negative. Newborn screening of metabolic disorders was performed due to existing of refractory vomiting and abdominal pain, the absence of drugs or toxic agents ingestion, history of brother death. Due to diagnosis of isovaleric acidemia on tandem mass metabolic screening panel we made IVD gene whole exon sequence analysis. As a result we determine the patient p.E117K(c.349G>A)(Homozygous). The analysis of known mutations that are sent from the patient's parent and his healthy siblings were determined as p.e117k (c.349G>A)(heterozygous). We present this case because it is a new mutation in the IVD gene.

#### E-P06.27

##### The PAH mutations in different ethnic groups from Rostov region (Russia)

M. A. Amelina<sup>1</sup>, S. S. Amelina<sup>2</sup>, A. A. Stepanova<sup>3</sup>, A. V. Polyakov A.V<sup>3</sup>, S. I. Kutsev<sup>3,4</sup>, R. A. Zinchenko<sup>3,4</sup>;

<sup>1</sup>Academy of Biology and Biotechnology of Southern Federal University, Rostov-on-Don, Russian Federation, <sup>2</sup>The Rostov State Medical University 29, Nakhichevansky Street, Rostov-on-Don, 344022, Russia, Rostov-on-Don, Russian Federation, <sup>3</sup>Research Centre for Medical Genetics, 115478, Russia, Moscow, Moskvoorechye st.1, Moscow, Russian Federation, <sup>4</sup>Pirogov Russian National Research Medical University, 117997, Russia, Moscow, Ostrovityanova st. 1, Moscow, Russian Federation.

Introduction: The PAH mutations in PKU patients of different ethnic groups from Rostov region were studied.

Materials and methods: Epidemiological, PCR, sequencing, MLPA. DNA analysis of PAH gene was conducted in 130 PKU patients (72.2% Russian, 3.95% Armenian, 3.95% Turks, 1.59% Dargin, 0.79% Jews).

Results: Diagnostic efficiency was 100%, 40 different PAH mutations were revealed. Among Russian PKU patients (190 chromosomes) the major mutation is R408W (50.77%). The second frequent mutation is IVS12+1G>A (3.46%). Less common mutations were R261Q(2.69%), P281L (1.54%), R158Q (2.31%), R252W(1.15%), L48S (1.15%), 21 mutations (A300S, EX-5del, IVS4+5G>T, IVS10-3C>T, R297H, A342T, A403V, E280K, F39del, F299C, IVS2+13T>G, IVS7+1G>A, IVS9+5G>A, IVS10-11G>A, K363fsdelG, R176X, R408Q, Y268C, p.N133\_Q134>Rfs, c.47\_48delCT, p.V245A) were detected only once.

The Armenians PKU patients (10 chromosomes) had genotypes: R252W/IVS10-11G>A (2 patients), F39del/IVS10-11G>A (1 patient), K363>Nfs/IVS10-11G>A (1 patient), P281L/R261Q (1 patient). The most frequent mutation was IVS10-11G>A and R252W.

The Meskhetian Turks PKU patients carried genotypes R408W/R408W (2 patients), IVS10-11G>A/IVS10-11G>A (2 patients) and E390G/IVS11+1G>C (1 patient).

In two Dargin PKU patients the major mutation was R261X, 3 of 4 chromosomes (genotype patients R261X/R261X, R261X/R261Q). The Jewish patient is a carrier of the genotype R408W/R408W.

Conclusions: The results of the study can be used for optimizing Medical Genetic Counselling in PKU families in the Rostov region.

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## E-P06.29

**Novel pathogenic variant in the PEX3 gene with unusual biochemical findings and a mild clinical presentation in the Zellweger syndrome spectrum**

K. Bjørgo<sup>1</sup>, H. H. Mørk<sup>1</sup>, R. Fjær<sup>1</sup>, S. Ferdinandusse<sup>2</sup>, S. S. Amundsen<sup>1</sup>, M. A. Kulseth<sup>1</sup>, K. Selmer<sup>1</sup>;

<sup>1</sup>Oslo University Hospital, Oslo, Norway, <sup>2</sup>Academic Medical Center, Amsterdam, Netherlands.

Zellweger spectrum disorders (ZSDs) are caused by a defect in peroxisome biogenesis due to mutations in one of 13 PEX genes. Clinical findings are heterogeneous, with the most important features being liver dysfunction, developmental delay and other neurological abnormalities, adrenocortical dysfunction and impairment of hearing and vision. Typically, ZSD patients accumulate very long-chain fatty acids, phytanic acid and bile acid intermediates, and have a deficiency of plasmalogens in erythrocytes. Most patients with a PEX3 defect present with a severe form of Zellweger syndrome with death in the first year of life.

We present a brother and sister from a consanguineous marriage who are homozygous for a novel pathogenic variant c.206-1G>T in intron 2 of PEX3. The patients are in their twenties and have spastic tetraparesis, nystagmus, visual impairment, leukodystrophy and mental retardation.

mRNA studies in patient fibroblasts revealed that the c.206-1G>T substitution abolishes the normal splice site. This leads to activation of a cryptic acceptor splice site and production of an in-frame transcript with a 27bp (9aa) deletion.

Biochemical studies revealed normal peroxisomal parameters in blood and fibroblasts, but catalase immunofluorescence microscopy analysis showed that peroxisomes were enlarged in most cells and in some cells the number of peroxisomes was reduced.

Our patients exemplify how a splice site variant predicted to give a severe phenotype can be less severe than expected due to activation of a cryptic splice site. Based on the mRNA studies we propose that the in-frame product has residual activity explaining the milder phenotype in our patients.

## E-P06.32

**Lack of association between rs1326634 polymorphism and T2D: Tehran Lipid and Glucose Study (TLGS)**

A. Zadeh-Vakili<sup>1</sup>, H. Karimi<sup>2</sup>, M. Zarkesh<sup>1</sup>, M. S. Daneshpour<sup>1</sup>;

<sup>1</sup>Cellular and Molecular Endocrine Research Center, Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of, <sup>2</sup>Department of biology, East Tehran branch, Islamic Azad University, Tehran, Iran, Islamic Republic of.

Introduction: Type 2 diabetes (T2D) is a multifactorial disease. One of the most important genes involving in this disease is SLC30A8. This study was conducted to investigate the relation between rs1326634 C>T polymorphism and T2D in a Tehranian population.

Materials and methods: Subjects were 603 T2DM and 1100 healthy, chosen from among participants of the Tehran Lipid and Glucose Study. Polymorphism from SLC30A8 gene was genotyped using the Tetra-Arms-PCR and results were confirmed by direct sequencing.

Results: Distributions of genotypes did not differ between cases and controls. The frequency of CC, CT, and TT genotypes was 29.4, 41.4, and 29.2% respectively, in controls. In the case group, the frequency of CC, CT, and TT was 24.6, 46.2, and 29.2%, respectively. Means of BMI, waist, and hip circumference were higher in TT control subjects ( $P < 0.05$ ). After adjustment for age, sex, and BMI in cases, the probability of high TG decreased by 54% among heterozygote (CT) vs. the homozygote combined group (CC+TT) (95% CI: 0.34-0.85,  $P=0.009$ ). After splitting by sex and adjustment for age and BMI, in male cases, the probability of high TG was significantly decreased by 47%, among heterozygotes (95% CI: 0.25-0.87,  $P=0.016$ ).

Conclusion: Although we found no association between the rs1326634 polymorphism and T2D, obesity-related indices, as risk factors for T2D, showed a significant relationship with TT genotype in controls. Based on these results, heterozygosity for T allele predisposes individuals to obesity, the effect that may be masked by other factors in diabetic patients.

## E-P06.36

**Identification of a new point mutation in MOCOS gene that is responsible for Xanthinuria Type II in a compound heterozygous case**

E. Perez, A. Lafuente, M. Ortiz, J. Orts, S. Ferrando, A. Zúñiga; Hospital de la Ribera, Alzira (Valencia), Spain.

Hereditary xanthinuria (HX) is a rare autosomal recessive disorder caused by a deficiency of xanthine dehydrogenase/oxidase (XDH/XO), affecting purine metabolism. Symptoms are related with extreme insolubility of xanthi-

ne, and include urolithiasis, crystalluria, hematuria, recurrent urinary tract infections and renal colic. Two clinically indistinguishable types of classical HX have been described. Type I (XDHD; OMIM#278300) is a simple XDH deficiency due to mutations in XDH gene, whereas Type II (XDHD/AO CD; OMIM603592) results from a combined deficiency of XDH and aldehyde oxidase (AO) caused by mutations in molybdenum cofactor sulfurase (MOCOS) gene, located on chromosome 18q12.2. Only about 150 cases of HX have been reported currently, being the prevalence higher in the Mediterranean and Middle East. Type I appears to predominate. We report a rare case of Type II HX in a female infant from non-consanguineous parents.

The patient presented hypouricemia and hypouricosuria, as well as higher concentrations of hypoxanthine and xanthine in urine. Xanthine calculi were confirmed by infrared spectroscopy. The genetic study was performed by PCR amplification of the XDH and MOCOS genes with specifically designed primers and subsequently Sanger sequencing. Results were negative for XDH, but two different mutations were found in MOCOS: c.1104\_1105delCT (p.Leu363ProfsTer16; NM\_017947) maternally inherited; and the novel mutation c.1387G>A (p.G463R; NM\_017947) paternally inherited. Pathogenicity was determined by *in silico* analysis with PolyPhen-2 and Mutation Taster softwares.

In our case, final diagnosis and typing of HX has been achieved by molecular genetics, avoiding more invasive tests like allopurinol loading test and intestinal or liver biopsy.

## E-P06.37

**Novel compound heterozygous mutation in zellweger syndrome**

S. Seyedhassani<sup>1,2</sup>, N. Tabibnejad<sup>1,3</sup>, M. Ebrahimi<sup>1</sup>;

<sup>1</sup>Dr. Seyedhassani Medical Genetic Center, Yazd, Iran, Islamic Republic of, <sup>2</sup>Genomic Research Center, Shahid Beheshti Medical Science University, Tehran, Iran, Islamic Republic of, <sup>3</sup>Research and Clinical Center for Infertility, Shahid Sadoughi Medical Science University, Yazd, Iran, Islamic Republic of.

Introduction: A couple with two neonates suffering from multiple congenital anomalies was referred to genetic counseling center. They had non-familial marriage without any evidence of genetic disorder in familial pedigree. The first neonate malformations were prominent forehead, low set ear, short neck, short nose, corneal clouding, micrognathia, high arch palate, pectus carinatum, ventricular septal defect, abnormal palmar crease, limbs deformities and club foot. Karyotype and CGH array were reported normal. He was died in third month. The second neonate had similar manifestations and was died in sixth month. The first case was assumed as Larsen syndrome before NGS. But the molecular analysis for FLNB gene was normal.

Materials and methods: Whole Exome Sequencing were used to enrich all exons of protein-coding genes as well as some important other genomic regions. Next generation sequencing was performed to sequence close to 100 million read on Illumina sequencer. In general, test platform examined >95% of the targeted regions with sensitivity of above 99%. Bioinformatics analysis of the sequencing results was performed using international databases and standard bioinformatics software. We performed Sanger sequencing on proband and parent in order to confirm the mutations.

Results: Two deleterious missense mutations in PEX1 gene were found. There were heterozygous condition NM\_001282677: exon21:c.3274dupC:p.Q1092fs and NM\_000466:exon7:c.G1407A:p.W469X in mother and father respectively.

Conclusions: There was no report of these mutations in the literature. Mutation in this gene is shown to cause Peroxisome biogenesis disorder 1A (Zellweger). This data can be used for genetic counseling and prenatal diagnosis.

## E-P07 Immunology and hematopoietic system

## E-P07.01

**Detection of Alpha Thalassemia by Using Multiplex Ligation Dependent Probe Amplification as an Additional Method for Rare Mutations in Southern Turkey**

O. Ozalp Yuregil<sup>1</sup>, A. Ayaz<sup>1</sup>, S. Yalcintepe<sup>1</sup>, S. Canbek<sup>1</sup>, D. Y. Acik<sup>2</sup>, B. T. Yilmaz<sup>3</sup>, T. B. Balci<sup>4</sup>;

<sup>1</sup>Numune Training and Education Hospital, Genetics Diagnosis Center, Adana, Turkey,

<sup>2</sup>Numune Training and Education Hospital, Department of Hematology, Adana, Turkey,

<sup>3</sup>Numune Training and Education Hospital, Department of Pediatric Hematology, Adana, Turkey, <sup>4</sup>Children's Hospital of Eastern Ontario, Department of Genetics, Ottawa, ON, Canada.

Alpha thalassemia is the most common single gene disorder in the Cukurova Region in Turkey. It is therefore routinely screened, including premaritally, in our region. The heterogeneous molecular basis of the disease makes al-

pha thalassemia mutation detection difficult and complex. Besides well established methods, multiplex ligation dependent probe amplification (MLPA) is known as an effective, simple and specific method for the detection and characterization of deletions and duplications. We employed MLPA testing to 30 patients with hematological parameters suggestive of alpha thalassemia carrier status but was negative for alpha thalassemia with conventional reverse dot blot hybridization (RDB). We found alpha globin gene deletions in 3 out of 30 (10 %) patients with MLPA. We propose that MLPA can be used as a second tier test in addition to other techniques such as RDB to identify alpha thalassemia carriers in high prevalence regions such as ours, thereby allowing clinicians to provide accurate genetic counselling.

#### E-P07.02

#### Role of the C1858T polymorphism of protein tyrosine phosphatase non-receptor type 22 (PTPN22) in children and adolescents with type 1 diabetes

*M. Provenzano, A. Blasetti, C. Di Giulio, S. Tumini, L. Comegna, G. Prezioso, R. Chiuri, F. Chiarelli, L. Stuppa;  
G d'Annunzio University, Chieti, Italy.*

Type 1 diabetes (T1D) is an immune-mediated disease causing the destruction of insulin-producing  $\beta$ -cells. The degree of  $\beta$ -cell destruction and the consequent amount of residual  $\beta$ -cell function are heterogeneous and can lead to variations in C-peptide secretion. In recent years, interest has been expressed on the susceptibility polymorphisms of T1D. The aim of this study was to define the frequency of the C1858T polymorphisms in the PTPN22 gene, which codes for a protein (Lyp) that may negatively regulate the proximal signaling pathway after T cell receptor activation, in a cohort of 113 Caucasian patients (58 male and 55 female) with T1D, and to assess a possible link to a group of clinically relevant variables: age at onset, gender, diabetes-related autoantibodies, residual  $\beta$ -cell function, and daily insulin requirement six months after diagnosis. The C1858T variant (rs2476601) was investigated through PCR-RFLP and we found that the frequency of the PTPN22 C1858T polymorphism in the analyzed diabetic patients is 17.7%. A statistically significant correlation between the polymorphism and higher levels of C-peptide at diagnosis and lower insulin requirement at six months from diagnosis was seen ( $p=0.001$  and  $p=0.04$ ). Moreover 1858T variant carriers were more frequently positive for glutamic acid decarboxylase autoantibodies than wild type subjects ( $p=0.19$ ) at diagnosis.

In conclusion, these findings if extended to a larger cohort of samples may characterize a subset of T1D patients with a defined genetic pattern, who may be eligible for trials aimed at preserving the residual  $\beta$ -cell function.

#### E-P07.03

#### A novel missense MVK mutation in a family with familial Mediterranean fever-like disease

*I. Karacan<sup>1</sup>, S. Uğurlu<sup>2</sup>, A. Tolun<sup>3</sup>, E. Tahir Turanlı<sup>1,4</sup>, H. Özdogan<sup>2</sup>;  
<sup>1</sup>Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey, <sup>2</sup>Department of Internal Medicine, Division of Rheumatology, Cerrahpaşa Faculty of Medicine, Istanbul University, Istanbul, Turkey, <sup>3</sup>Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey, <sup>4</sup>Dr. Orhan Ocalgiray Molecular Biology-Biotechnology and Genetics Research Center, Istanbul Technical University, Istanbul, Turkey.*

**Introduction:** Monogenic autoinflammatory diseases are mainly disorders of innate immunity and characterized by unprovoked inflammatory attacks. We studied a consanguineous family with two affected children, with an initial diagnosis of Familial Mediterranean Fever (FMF). While recurrent febrile attacks with serositis, high acute response and parental origin were consistent with FMF, disease onset before age one year, delay in growth, no mutation in MEFV and poor response to colchicine were not typical features of this disease.

**Methods:** SNP genotype data for all family members were used for multi-point linkage analysis. Targeted sequencing was performed for MVK residing in one of the linked regions and seven other autoinflammation related genes, IL1RN, LPIN2, MEFV, NLRP12, NLRP3, TNFRSF1A and PSTPIP1. After the identification of the causative mutation, serum IgD and urinary mevalonic acid levels were measured.

**Results:** Linkage analysis detected seven candidate regions. The largest candidate region, at 12q24.11-q24.31 (LOD=1.92), contained 191 genes. Novel homozygous c.481T>C (p.Cys161Arg) mutation in MVK, the gene responsible for Hyper IgD Syndrome (HIDS) was identified. At the protein level, cysteine at position 161 is totally conserved in mammals. Urinary mevalonic acid was not detected for either patient, and serum IgD level was slightly elevated for only one patient.

**Conclusions:** We identified a novel homozygous MVK mutation in patients with a FMF-like disease but without a typical HIDS phenotype. We hypothe-

size that this novel mutation underlies the atypical clinical presentation. Phenotypic variability of HIDS is well known, and our findings further expand the MVK mutation phenotype.

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#### E-P07.05

#### EKLF mutation co-related with HPFH phenotype in $\beta$ -thalassemia patients in Iran

*M. Hamid<sup>1</sup>, S. Ershadi Oskouei<sup>2</sup>, G. Shariati<sup>3</sup>, E. Babaei<sup>2</sup>, H. Galehdar<sup>3</sup>, A. Saberi<sup>3</sup>;  
<sup>1</sup>Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of, <sup>2</sup>University of Tabriz, Tabriz, Iran, Islamic Republic of, <sup>3</sup>Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, Islamic Republic of.*

**Introduction:** KLF1 gene is an essential transcription factor for primitive and definitive erythropoiesis, any mutation in KLF1 gene may interfere with its proper related function in erythropoiesis process in red blood cell maturation and lead to alter proper activation of its downstream protein through globin switching, which results an increase in fetal globin blood level (HbF). This study aims to investigate whether KLF1 mutation can associate with high HbF level in  $\beta$ -thalassemia patients in southwest of Iran, the region that has high frequency of hemoglobinopathy disorders. **Materials and Methods:** At first the human KLF1 gene was amplified via PCR procedure, and sequencing was used to determine any mutation in these patients. Also XmnI polymorphism in the position of -158 of  $\gamma$ -globin gene promoter were analyzed in all patients. **Results:** Analysis of sequencing revealed a missense mutation in KLF1 gene, S102P, which was detectable in 10 out of 23 cases with HPFH phenotype. Among studied population only in individuals with HbF level between 3.1%–25.6% the mutation was identified. Statistical analysis showed that allele frequency of the mutation S102P among studied group is significant (P-Value=0.3383). Allele frequency for positive and negative results of XmnI digestion in patient with increased HbF level 0.565, and 0.434 were determined respectively, which showed a positive association with increased HbF level (P-Value= 0.3508). **Conclusions:** According to statistical results of S102P mutation, and Xmn1 Polymorphism strongly suggest that both polymorphisms have association with HPFH phenotype in southwest of Iran.

#### E-P07.07

#### Combined immunodeficiency in 11q terminal deletion (Jacobsen) syndrome

*T. Hovnik<sup>1</sup>, A. Ihan<sup>2</sup>, L. Lovrečić<sup>3</sup>, Š. Blazina<sup>4</sup>;*

*<sup>1</sup>Unit of Special Laboratory Diagnostics, Children's Hospital Ljubljana, University Medical Centre Ljubljana, Slovenia, Ljubljana, Slovenia, <sup>2</sup>Institute of Microbiology and Clinical Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, Ljubljana, Slovenia, <sup>3</sup>Clinical Institute of Medical Genetics, Division of Obstetrics and Gynecology, University Medical Centre Ljubljana, Slovenia, Ljubljana, Slovenia, <sup>4</sup>Department of Allergy, Rheumatology and Clinical Immunology, Children's Hospital Ljubljana, University Medical Centre Ljubljana, Slovenia, Ljubljana, Slovenia.*

**Background:** Terminal 11q deletion syndrome also known as Jacobsen syndrome (JS; OMIM#147791) is a rare genetic disorder that affects different body systems. Antibody deficiency is common in patients with JS but there has been only a few reports on additional T-cell defects.

**Methods and results:** Standard and molecular cytogenetics studies on peripheral blood lymphocytes from patient and her parents showed 46,XX,del(11)(q23.3).ish del(11)(q23.3)(D11S1037-)dn. Using array CGH, deletion breakpoint was shown to be at the band 11q24.1 encompassing most of the crucial genes responsible for typical phenotype of JS. Immunologic workup was consistent with antibody deficiency. In addition to low IgM, IgG4 and B-cells, low recent thymic emigrants, helper and naïve T-cells were also found.

**Conclusion:** We present a patient with confirmed JS in whom T-cell deficiency was observed in addition to antibody deficiency and other features of the syndrome. T-cell deficiency was not present during the initial evaluation, which highlights the importance of performing a thorough immunological follow up in patients with 11q terminal deletion syndrome. The prevalence of combined immunodeficiency in patients with JS is not known, as most patients have not yet been evaluated for T-cell defect.

#### E-P07.10

#### Vitamin D genes CYP27B1 and VDR regulated by the MS functional variant rs1087713 under inflammatory and autocrine-like stimuli

*M. Fedetz<sup>1</sup>, M. Karaky<sup>1</sup>, C. Barrionuevo<sup>1</sup>, C. Delgado<sup>2</sup>, G. Izquierdo<sup>3</sup>, A. Alcina<sup>1</sup>, F. Matesanz<sup>1</sup>;*

*<sup>1</sup>Instituto de Parasitología y Biomedicina López Neyra, CSIC, Granada, Spain, <sup>2</sup>Centro Regional de Transfusión Sanguínea Granada-Almería (CRTS), Granada, Spain, <sup>3</sup>Unidad*

de Esclerosis Múltiple, Hospital Universitario Virgen Macarena, Sevilla, Spain.

**Introduction:** Vitamin D deficit is considered an important risk factor for many inflammatory and autoimmune diseases. In this study we present the influence of multiple sclerosis-associated regulatory variant rs10877013 on the expression of genes involved in vitamin D activation (CYP27B1), vitamin D receptor (VDR) and vitamin D degradation (CYP24A1) under inflammatory environment.

**Materials and Methods:** We used lipopolysaccharide and interferon gamma activated monocytes from 119 individuals and vitamin D-stimulated lymphoblastoid cell lines (LCLs, n=109) of 1000 Genomes to quantify the mRNA expression of vitamin D genes by RTqPCR.

**Results:** The expression level of CYP27B1 was associated with the rs10877013 genotypes ( $P= 5.0E-6$ ) in activated monocytes. This association between gene expression and variant genotypes was not observed in LCLs. Inversely, rs10877013 genotypes were associated with VDR expression in LCLs ( $P= 6.0E-4$ ) but it was not in monocytes. Finally, CYP24A1 was highly and specifically induced by active vitamin D (1,25-(OH)2D3) and its expression correlated with the expression of VDR in LCLs but no polymorphism was found to be associated with its expression in a search of variants in 1 Mb around its position.

**Conclusions:** The MS-associated variant rs10877013 is a genetic determinant that affects the functioning of vitamin D system linking environmental and genetic risk factors.

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#### E-P07.11

##### A Case of Novel Translocation

*M. N. Somuncu, A. G. Zamani, M. S. Yıldırım;*

*Department of Medical Genetics, Meram Medical Faculty, Necmettin Erbakan University, KONYA, Turkey.*

Translocations are the rearrangement when a segment from one chromosome becomes a nonhomologous chromosome or to a new site on the same chromosome. Genetic changes especially structural chromosomal aberrations as translocations have been usually observed in hematologic malignancy. Pure red cell aplasia (PRCA) is an hematologic disorder that maturation of erythrocytes are arrested and erythroblasts are mostly absent in bone marrow however white blood cell (WBC) and platelet production is normal. Ethiopathogenesis of PRCA is clinic heterogeneity but the anemia usually can cause to PRCA and red blood cell (RBC) is ceased to produce in bone marrow. So that the maturation and differentiation of the blood cells can be hinder in hematologic pathway and can even lead to childhood lymphoma. Structural rearrangement of chromosome 1q31 between chromosome 2q37 has been reported with PRCA ten years old patient. Proband were referred to our clinic for bone marrow karyotype analise had thrombocytopenia and anemia phenotype from pediatric hematology. We analysed the patient karyotype as 46,XX t(1;2)(q31;q37).

We have reported this novel rearrangements that has not been observed in any disorder contributing to the literature.

#### E-P07.12

##### Thrombomodulin gen analysis in a case of atypical haemolytic-uremic syndrome

*I. Tirado<sup>1</sup>, N. Vilalta<sup>1</sup>, J. Mateo<sup>1</sup>, I. Coll<sup>1</sup>, B. Cuevas<sup>1</sup>, J. López-Pardo<sup>1</sup>, G. Fraga<sup>2</sup>, S. Brió<sup>2</sup>, E. Martínez-Sánchez<sup>1</sup>, J. Fontcuberta<sup>1</sup>;*

*<sup>1</sup>Thrombosis and Haemostasis Unit, Hematology Department Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>2</sup>Pediatrics Department Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.*

**Introduction:** Atypical haemolytic-uremic syndrome (HUS) is a condition presenting with the classic triad (microangiopathic haemolytic anaemia, thrombocytopenia and renal failure). Half of the patients have mutations in genes that regulate the complement system. Recently, mutations in the thrombomodulin (THBD) gene (an endothelial protein with anti-inflammatory and anticoagulant properties) have been reported in 5% cases. Our laboratory investigated the thrombomodulin gene in a patient diagnosed with atypical HUS.

**Materials and Methods:** A 21 month- old female was admitted to our emergency room with 5 days evolution fever. Physical examination revealed micropetechiae at lower limbs and hepatomegaly. Laboratory parameters showed microangiopathic haemolytic anaemia, acute renal failure, thrombocytopenia, hyperferritinemia, hypofibrinogenemia, elevated D-dimer concentration and 9% of ADAMTS-13 activity. With all these criteria the patient was diagnosed of atypical HUS and macrophagic activation syndrome.

We investigated the exon 1 of thrombomodulin gene associated with the atypical HUS. ABI3500 (Applied Biosystems®) was used to Sanger sequence the regions of the gene in different fragments delimited by primers that included all exon region of thrombomodulin gene.

**Results:** No mutations were found in the fragments sequenced of the patient. In our patient, DNA fragments from thrombomodulin gene analysed by direct sequencing do not show any variation. We performed the same analysis in a control group without findings.

**Conclusion:** The patient here discussed was diagnosed with atypical HUS and did not present THBD mutations. Next step will be study other genes implicated in this syndrome.

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#### E-P07.13

##### Association between CARD15/NOD2 gene polymorphisms and Ulcerative colitis (UC) and Crohn's disease in Turkish population

*S. Budak Diler<sup>1</sup>, E. Derici Eker<sup>2</sup>, F. Polat<sup>3</sup>, S. Yaraş<sup>2</sup>;*

*<sup>1</sup>Niğde University, Niğde, Turkey, <sup>2</sup>Mersin University, Mersin, Turkey, <sup>3</sup>Kocaeli University, Kocaeli, Turkey.*

**Introduction:** Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel diseases which are similar to each other for their clinical features and epidemiology. R702W, G908R and 1007fsInsc polymorphisms in CARD15/NOD2 genes are found to be related to this disease. We aimed to determine P268S and M863V polymorphisms in CARD15/NOD2 genes associated with an increased risk of developing UC and CD and to establish correlations between P268S and M863V genotypes in Turkish population.

**Materials and Methods:** This study included 153 healthy controls and 107 patients (42 CD and 65 UC) with CD and UC. The P268S and M863V gene regions were amplified using polymerase chain reaction (PCR), detected by restriction fragment length polymorphism (RFLP). The results of patient and control group were evaluated statistically.

**Results:** According our results, the P268S CC genotype was prevalent on patients and controls (55% vs 67%), followed by genotypes CT (38% vs 27%) and TT (7% vs 6%) in CD. The prevalence of genotypes of CC (wild-type), CT (heterozygous mutant) and TT (homozygous mutant) profiles for the P268S polymorphism were 69%, 25% and 6% respectively in UC patients, and 67%, 27% and 6% respectively in healthy control groups. Just two bands of 212 bp and 160 bp were found in wild-type M863V in all subjects and no other mutant band (372 bp) of M863V was detected using PCR-RFLP fragment electrophoresis.

**Conclusions:** There are no association P268S and M863V polymorphisms between patients with CD and UC and control groups in Turkish population.

#### E-P08 Intellectual Disability

##### E-P08.01

##### Global developmental delay in a girl with Int22h1/int22h2-mediated Xq28 deletion.

*E. Lloveras, L. Barranco, V. Cirigliano, A. Canellas, M. Costa, C. de la Iglesia, D.*

*Fernandez, M. Herrero, B. Méndez, N. Palau, M. Piqué, D. Yeste, E. Ordóñez, L. Rueda, A.*

*Plaja;*

*Departments de Genética i Genètica Molecular. LABCO Iberia. Barcelona, Esplugues de Llobregat, Spain.*

**Introduction:** A new deletion/duplication syndrome within Xq28 chromosomal band has recently been described caused by nonallelic homologous recombination between low-copy repeats (LCRs) intron 22 homologous region 1 (int22h1) and 2 (int22h2), in addition to int22h3, within the F8 gene. The duplication has been identified in males with cognitive impairment and females with mild or normal phenotype. The reciprocal deletion is rare. Only few cases have been described in the literature of normal females with skewed inactivation and at higher risk of miscarriages. We report a case of a girl with global developmental delay and Int22h1/int22h2-mediated Xq28 deletion.

**Material and methods:** ArrayCGH (Cytochip ISCA 8X60K, Bluegnome, Illumina) was performed in peripheral blood from a 4 years old girl with global developmental delay. Her mother had clinical history of miscarriages.

Chromosome X inactivation studies were performed in peripheral blood using methylation-specific PCR of the polymorphic CGG repeat in the promoter region of the FMR1 gene.

**Results:** The proband was found to have a 450kb Xq28 deletion spanning from 154.1 to 154.5 Mb. The deletion included the F8 [MIM 300841], RAB39B [MIM 300774], CLIC2 [MIM 300138] and VBP1 [MIM 300133] genes. Complete skewed X chromosome inactivation pattern was observed.

The history of recurrent miscarriage reported in the mother strongly suggests her being an asymptomatic carrier of the same deletion. ArrayCGH is in progress.

Conclusions: Our patient shows global developmental delay and Int22h1/int22h2-mediated Xq28 deletion suggesting, but not demonstrating, the possibility that female carriers may occasionally show global developmental delay.

## E-P08.03

### Microdeletion of 10q21.3-q22.1 including CTNNA3 in a boy with intellectual disability, mucocutaneous pigmentation, and juvenile colon polyposis

C. Venegas-Vega<sup>1,2</sup>, M. Guardado<sup>1</sup>, J. Berumen<sup>1,2</sup>;

<sup>1</sup>Hospital General de Mexico, Mexico DF, Mexico, <sup>2</sup>Facultad de Medicina, Universidad Nacional Autónoma de México, UNAM, Cdmx, Mexico.

Introduction: We described the case of a 9-year-old boy with a de novo 10q21.3-q22.1 microdeletion, intellectual disability (ID), attention-deficit hyperactivity disorder, mucocutaneous pigmentation, and juvenile colon polyposis (JCP). Methods and Results: Cytogenetic analyses in the proband and her parents was normal; Chromosomal Microarray Analysis (CMA) by CytoScan Optima in the patient revealed a deletion of about 4.41 Mb ([hg19] chr10:68,299,927-72,715,960), while CMA of parental DNA was normal. Although no similar size deletions were reported in DECIPHER database, there are several patients with much smaller pure deletions (86.05 to 378.77 Kb) that clustered at CTNNA3 gene (chr10:68,165,545-69,222,108). The most remarkable common feature of these cases was intellectual disability, global developmental delay and autism spectrum disorder. Moreover, the analysis of the gene content of the deleted region showed 40 MIM genes; of which only four have been implicated in autosomal dominant diseases. Our patient does not exhibit any clinical data of those conditions. In order to identify that genes could be involved in the phenotype, we analyzed physiologic roles, functional interactions, and also which those are dosage-sensitive. The results pointed to CTNNA3 (607667), LRRTM3 (610869) and SIRT1 (604479); all located in 10q21.3, as best candidates. Thus, these three genes may either individually or in combination be responsible for ID with JCP seen in our patient. Conclusions: To the best knowledge, this is the first report of a de novo 10q21.3-q22.1 deletion. This case suggests a novel genomic disorder at 10q21.3 region associated to ID with JCP.

## E-P08.04

### Different terminal chromosome 12q microdeletions are associated with defined clinical features

S. Genovese<sup>1</sup>, V. Alesi<sup>1</sup>, C. Surace<sup>1</sup>, M. Bertoli<sup>2</sup>, M. Digilio<sup>3</sup>, A. Novelli<sup>1</sup>;

<sup>1</sup>Laboratory of Medical Genetics, "Bambino Gesù" Children's Hospital, Rome, Italy,

<sup>2</sup>Institute of Genetic Medicine, Newcastle University - Central Parkway, Newcastle upon Tyne, United Kingdom, <sup>3</sup>Medical Genetics Unit, "Bambino Gesù" Children's Hospital, Rome, Italy.

Introduction: Deletion of 12q telomere is rare and few cases with different breakpoints and clinical features have been described in literature so far.

Materials and methods: We have studied by means of oligo array CGH (Comparative Genomic Hybridization) two cases with this rearrangement: a 12 years old female patient and a 13 years old male patient both with intellectual disability, behavioral disorder, and obesity.

Results: We have detected two microdeletions in the 12q24.33 chromosome region, 3.4 Mb and 1.4 Mb in size respectively, involving many common genes that may contribute to the clinical picture of the patients. These microdeletions partially overlap with deletions of other patients described in DECIPHER (Database of genomic variation and Phenotype in Humans using Ensembl Resources; <https://decipher.sanger.ac.uk/>) and previously published in literature with intellectual disability, kidney disease and problem of development of genitals. We have observed the presence of a minimum deleted region (660 Kb), located in the 12q subtelomeric segment including the P2RX2 gene, which has been previously proposed as a candidate gene for intellectual disability. In fact, behavioral disorder and mild mental retardation were common to all the patients. Larger deletions extended up to the proximal regions (3-9 Mb) containing the STX2 and NCOR2 genes, seem to be involved in urinary-genital apparatus and connective tissue anomalies.

Conclusion: The comparison between our patients and the other ones already described has allowed us to link the size of deleted region to the clinical features.

## E-P08.05

### 17p13.1 microdeletion: genetic findings on microcephaly

E. A. Rolo, A. Sousa, S. Serafim, R. Rodrigues, S. Custódio, R. Silveira-Santos, J. Dupont, A. B. Sousa;

Serviço de Genética Médica, Departamento de Pediatria, Centro Hospitalar Lisboa Norte-Hospital Santa Maria, EPE; Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Introduction: 17p13.1 microdeletion syndrome has a wide spectrum of clinical features, including intellectual disability, absent/very poor speech, dysmorphism and microcephaly. This clinical diversity is partly a consequence of deletion size, which ranges from 245kb to 4.4Mb. Thus, it is suggested that two or more microdeletion syndromes may exist within this region and a genotype-phenotype correlation has yet to emerge. Here we discuss the genetic and clinical features observed in a patient with a small 17p13.1 deletion. We focus on microcephaly, a prominent feature of this syndrome. Material and Methods: We report a 4-year-old boy referred to aCGH testing presenting with global psychomotor delay, facial dysmorphisms and microcephaly. Family history was unremarkable. Samples were studied by aCGH (NimbleGen CGX 135K, Perkin Elmer). Genomic analysis was performed with Genoglyphix v3.0 software (Signature Genomics). Results: aCGH revealed a de novo microdeletion of 287.53kb in the 17p13.1 region (hg19; chr17:7,119,830-7,407,357) containing 23 OMIM genes. Overlapping our deletion with the previously reported 17p13.1 microdeletions, we identified a new minimal region of 92kb for microcephaly. Conclusions: The phenotype observed in our patient is consistent with the 17p13.1 microdeletion syndrome. Previously reported patients with 17p13.1 deletions pointed to an overlapping region associated with microcephaly, including four genes, ASGR1, ACADVL, DVL2 and GABARAP. Considering the presence of microcephaly in our patient and the lack of ASGR1 gene in the deleted segment, we propose to exclude this gene as a candidate for this feature. These findings contribute to narrow the 17p13.1 critical genomic region associated with microcephaly.

## E-P08.06

### 1q44 microdeletion syndrome: a de novo 314 kb CNV involving HNRNPU and FAM36A genes in a girl with psychomotor delay, epilepsy and corpus callosum hypoplasia

A. Sousa, S. Custódio, R. Silveira-Santos, P. Dias, A. B. Sousa;

Serviço de Genética Médica, Departamento de Pediatria, Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Centro Académico de Medicina de Lisboa, Lisboa, Portugal.

Introduction: 1q44 terminal microdeletion has emerged as a clinically recognizable syndrome. Phenotypic presentation, although heterogeneous, includes moderate to severe developmental delay (DD)/intellectual disability (ID), microcephaly and seizures. Corpus callosum abnormalities are also commonly observed, but attempts at defining its critical region have yielded inconsistent results to date. Small size interstitial deletions within 1q44 locus are rare and help to appoint candidate genes for this particular trait.

Materials and Methods: We describe a 12-year-old girl referred for array-CGH testing due to moderate DD/ID. At physical examination, she presented short stature, craniofacial dysmorphisms including bifid uvula, convergent strabismus, bilateral ankle valgus deformity and pes planus; head circumference was normal. Brain-MRI revealed corpus callosum hypoplasia. She had been diagnosed with absence epilepsy at 11.

Results: A de novo 314 Kb deletion in 1q44 terminal region was detected by arrayCGH (CGX-HD 180K, Signature Genomics/Perkin Elmer), including the FAM36A, HNRNP and EFCAB2 genes (HG19 Chr1:244,961,796-245,276,463).

Discussion/Conclusions: Except for the absence of microcephaly, this patient has a full 1q44 microdeletion phenotype. Selmer KK et al. reported a boy with a 163 kb deletion in 1q44 involving the FAM36A and HNRNPU genes, who also had moderate DD/ID, absence seizures, thin corpus callosum and normal head circumference. This case reinforces FAM36A and HNRNPU as good candidate genes for 1q44 microdeletion features, namely corpus callosum abnormalities. Functional studies are needed to clarify the role of these genes in brain development.

## E-P08.07

### Case report compilation of submicroscopic chromosomal gains and losses at Xp22 region in individuals with intellectual disability detected by Microarray based comparative genomic hybridization (array-CGH)

S. Franco<sup>1</sup>, C. Benito<sup>1</sup>, V. A. Penacho<sup>2</sup>, I. Manchón<sup>2</sup>, D. Amorós<sup>2</sup>, I. Gómez-Milánés<sup>2</sup>, L. A. Alcaraz<sup>2</sup>;

<sup>1</sup>Hospital Regional Málaga, Málaga, Spain, <sup>2</sup>Bioarray, S.L., Elche, Spain.

**Context:** Submicroscopic chromosomal gains and losses are responsible for intellectual disability (ID) and dysmorphic features that affects about 3% of the population. The distal portion of the short arm of the human X chromosome (Xp22) is a region that undergoes frequent genomic rearrangements. **Objective:** Our goal is to compile case reports of submicroscopic gains and losses located at Xp22 region that were undetectable by routine cytogenetic analysis while reliable detection was feasible by array-CGH. **Methods:** Samples from 14 subjects anonymized (5 unrelated), collected at Hospital Carlos Haya, were ascertained in Bioarray as having Xp22 gains/losses. Five unrelated patients presented ID and dysmorphism suspicious of an underlying chromosome abnormality not confirmed by karyotyping. Agilent 60K Oligonucleotide array-CGH platform based on the construction of the hg19 genome sequence was used in order to detect such gains/losses. **Results:** Five unrelated cases carried copy number variations ranging in size from ~0.126 to ~1.6 Mb. Two subjects showed duplications of maternal origin (microduplication at Xp22.31) and three had submicroscopic changes of unknown origin, as parental testing of origin was not performed (microdeletions at Xp22.33 and Xp22.32, and microduplication at Xp22.33). DLRs value that calculates the probe-to-probe log ratio noise of arrays was <0.2 in all the cases, which means a precise detection of very small aberrations. **Conclusions:** Our data confirm that Agilent 60K array-CGH can accurately detect microduplications and microdeletions at Xp22 region. It contains over 60,000 probes designed especially to detect small gains/losses in regions associated with mental retardation and/or developmental syndromes, among others.

#### E-P08.08

#### Implementation of aCGH assessment in genetic diagnostic of patient with global development delay/intellectual disability or multiple congenital anomalies - Romanian experience

I. Streata, S. Sosoi, A. L. Riza, F. Burada, F. Mixich, M. Ioana;

University of Medicine and Pharmacy of Craiova, Human Genomics Laboratory, Craiova, Romania.

**Introduction:** Assessment of DNA copy number through array-CGH represent a powerful diagnostic tool for clinicians. Genomic microarrays are used as first-tier genetic tests in postnatal assessment of subjects diagnosed with unexplained global development delay (GDD)/ intellectual disability (ID), autism spectrum disorders (ASD) or multiple congenital anomalies (MCA) in almost all West European countries. Two years ago, we started to implement in South-West Romania the postnatal aCGH assessment of individuals with unexplained GDD/ID or CMA.

**Materials and methods:** We assessed 30 patients diagnosed with unexplained GDD/ID or CMA. Conventional cytogenetic evaluation did not detect any chromosomal imbalance that could be associated with the clinical phenotype of the patients. DNA isolated and purified from peripheral blood was examined for copy number variations (CNVs) using Roche NimbleGen 3x720K/12x135K or Agilent 4x180K/ 8x60K ISCA design oligonucleotide microarray. Copy number data was analyzed with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA).

**Results:** aCGH assessment identified in several patients submicroscopic changes (microdeletions or microduplications) that could partially explain the clinical phenotype.

**Conclusion:** This study demonstrated the utility and importance of genetic testing through aCGH in the management and counseling of patients with unexplained GDD/ID or CMA and their families.

#### E-P08.10

#### Complete genetics study of a Bardet-Biedl Syndrome: from 60K oligonucleotide array-CGH to whole-exome sequencing

C. Benito<sup>1</sup>, S. Franco<sup>1</sup>, V. Penacho<sup>2</sup>, D. Amorós<sup>2</sup>, I. Manchón<sup>2</sup>, I. Gómez-Milánés<sup>2</sup>, L. A. Alcaraz<sup>2</sup>;

<sup>1</sup>Hospital Regional Málaga, Málaga, Spain, <sup>2</sup>Bioarray, S.L., Elche, Spain.

**Context:** The Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by retinitis pigmentosa (RP), obesity, polydactyly, hypogonadism, kidney dysfunction and cognitive impairment. Its diagnosis is established by clinical findings and pathogenic variants found in at least 19 genes. **Case Report:** Four-year-old boy had development delay, hypogenitalism, obesity and poor visual acuity. He was born with polydactyly for which he had surgery at 10-month-old. He also presented polycystic kidney disease, RP and hypospadias. No family history was reported. A Bardet-Biedl syndrome was suggested at Hospital Carlos Haya. **Objective:** Genetics study by CGH+SNP microarrays and whole-exome sequencing to identify pathogenic BBS-related variants. **Methods:** Blood samples from boy and both parents were ascertained in Bioarray. Firstly, 129 BBS-related mutations were studied by Agilent Custom CGH+SNP microarray. Both Agilent 60K and 400K

array-CGH were performed to detect microduplications/microdeletions. Finally, a whole-exome sequencing (Illumina HiSeq 2000) was carried out. **Results:** A total of 115/129 BBS-related mutations analyzed were excluded; 14 of them, in BBS1 gene, got no signal. Array-CGH 60K detected a microdeletion that affects one probe in BBS1 so size cannot be determined due to its low-density. No loss of heterozygosity was shown by CGH+SNP Microarray. Results from array-CGH (400K) determined a 11q13.2 homozygous microdeletion in the boy that affects BBS1 (0.011 Mb). Also, heterozygous deletions in both of the asymptomatic parents were found. Whole-exome sequencing confirmed BBS1 deletion and its size. **Conclusion:** Our data confirm that combination of Agilent 400K array-CGH and whole-exome sequencing can accurately detect pathogenic BBS-related variants.

#### E-P08.11

#### Microarray analysis for novel copy number variations in Saudi family with intellectual disability and epilepsy

M. I. Naseer<sup>1</sup>, A. G. Chaudhary<sup>1</sup>, M. Rasool<sup>1</sup>, F. Talal Ashgan<sup>1</sup>, H. Schulten<sup>1</sup>, F. Bibi<sup>2</sup>, M. M. Jan<sup>3</sup>, M. H. Al-Qahtani<sup>1</sup>;

<sup>1</sup>CEGMR, King Abdulaziz University, jeddah, Saudi Arabia, <sup>2</sup>KFMRC, King Abdulaziz University, jeddah, Saudi Arabia, <sup>3</sup>Department of Pediatrics, Faculty of Medicine, King Abdulaziz University, jeddah, Saudi Arabia.

#### Background

Epilepsy is genetically complex but common brain disorder affecting millions of people of the world with almost of all age groups. Novel Copy number variations (CNVs) are considered as important reason for the numerous neurodevelopmental disorders along with intellectual disability and epilepsy.

#### Material and Methods

In order to study novel CNVs and related genes in Saudi family in six affected and two normal individuals with epileptic seizures, intellectual disability (ID), and minor dysmorphism, we performed the high density whole genome Agilent sure print G3 Hmn CGH 2x 400K array-CGH chips analysis results were also validated by using quantitative real time PCR.

#### Results

Our results showed novel deletions, duplications and deletion plus duplication on differential chromosomal regions in the affected individuals that were not shown in the normal and parents. Amplifications were observed in the chromosome 1, 16 and 22 with LCE3C, HPR, GSTT2 and GSTTP2 genes respectively whereas the deletions were observed in the chromosomal regions 8p23-p21, the potential gene in this region is CSMD1 (OMIM: 612279) and chromosome 11 with potential genes OR4C6 and OR4P4 respectively.

#### Conclusions

We found some of the novel deletions and duplication in our study. Our results suggest that array-CGH should be used as a first line of genetic test for epilepsy except there is a strong indication for a monogenic syndrome. The aim of this study was to identify novel mechanisms underlying epileptic disorder, may help to recover the clinical management of individual cases in decreasing the burden of epilepsy in Saudi Arabia.

#### E-P08.12

#### Complex chromosomal rearrangement - terminal deletion 21q22.13, duplication 4p16.2, and putative mosaic trisomy 8 - in a child with developmental delay, mild facial dysmorphic signs, and muscle hypertonia

U. Heinrich<sup>1</sup>, D. Wahl<sup>2</sup>, M. Heinrich<sup>3</sup>, I. Rost<sup>1</sup>;

<sup>1</sup>Centre for Human Genetics and Laboratory Diagnostics, Martinsried, Germany,

<sup>2</sup>Medical Practice for Genetic Counseling and Psychotherapy, Augsburg, Germany, <sup>3</sup>II. Children's Hospital of the Central Hospital, Augsburg, Germany.

**Case report:** Here we present a 7 months old female with facial dysmorphic signs, high palate, hypotonia, pes supinatus, short stature, and borderline microcephaly. She is the first child of healthy nonconsanguineous parents.

**Materials and Methods:** Conventional karyotyping was performed using standard GTG banding technique. For array CGH analysis the CytoChip Oligo 180K microarray was used. Segregation analyses were performed by quantitative PCR. For the validation of the putative mosaic trisomy 8, FISH analysis was carried out on peripheral blood cell nuclei using a centromere 8 probe. **Results:** Karyotyping of peripheral blood lymphocytes revealed a terminally deleted chromosome 21. The characterization using array CGH showed a 9,22 Mb deletion with the proximal breakpoint lying in the DYRK1A gene in band 21q22.13. In addition, a 355 kb duplication in band 4p16.2 including the genes EVC and EVC2 as well as a putative mosaic trisomy 8 was revealed. Parental karyotyping was inconspicuous proving the deletion 21q to be de novo, and qPCR analyses could demonstrate the maternal origin of the duplication 4p16.2. FISH analysis of peripheral blood cells could not confirm

the putative mosaic trisomy 8.

**Discussion:** Pure partial monosomy 21q is a rare event and is associated with highly variable phenotype including variable dysmorphic signs, heart defects, seizures, motor developmental delay as well as intellectual disability. The influence of the maternally inherited duplication 4p16.2 on the phenotype remains unclear. As the putative mosaic trisomy 8 could not be confirmed in blood cells, the examination of a second tissue should be considered.

## E-P08.13

### Interstitial deletion of 5q35.1-q35.2 detected by array-CGH in a boy presenting with heart defect and intellectual disability

**A. Röpke, C. Müller-Hofstede, S. Ledig, P. Wieacker, F. Tüttelmann;**  
Institut für Humangenetik, Münster, Germany.

We report on a 15 years old boy, first child of healthy, non-consanguineous parents. During pregnancy, a ventricular septal defect (VSD), atrial septal defect (ASD) and an omphalocele were diagnosed. At the time of presentation, he demonstrated global developmental delay and unspecific facial dysmorphism. Conventional cytogenetic analysis revealed an apparently normal male karyotype (46,XY; GTG banding at 550 band level). Array-CGH analysis (Agilent 400k microarray set) uncovered a de novo deletion of 1.6 Mb in the chromosomal region 5q35.1-q35.2. This deletion was confirmed by FISH analysis.

The deletion comprises 11 genes including the NKX2-5 gene (OMIM 600584). Mutations in the NKX2-5 gene were identified in patients with ASD, VSD, tetralogy of Fallot, and other congenital cardiac defects. The deletion does not overlap with the minimal Sotos syndrome deletion region. To the best of our knowledge, there are only two reports on patients with small deletions in 5q35.1-q35.2 including the NKX2-5 gene. One patient was described with severe heart defect, microcephaly, minor facial dysmorphic features, scoliosis, cryptorchidism, learning difficulties associated with ADHD and an IQ of 83. He carried a 2.2 Mb deletion in 5q35.1-q35.2 (Baekvad-Hansen et al., 2006). The other patient (Decipher database, patient 257414) with ASD, cryptorchidism, hypothyroidism and intellectual disability showed a 2.1 Mb deletion in 5q35.1-q35.2.

## E-P08.14

### Down syndrome and obstructive sleep apnea in children

**T. Marcovici<sup>1,2</sup>, M. Puiu<sup>1,2</sup>, G. Brad<sup>1,2</sup>, D. Nicoara<sup>2</sup>, T. Bizerea<sup>1</sup>, O. Marginean<sup>1,2</sup>,**  
<sup>1</sup>University of Medicine and Pharmacy „Victor Babes”, Timisoara, Romania, <sup>2</sup>Louis Turcanu“Children’s Emergency Hospital, Timisoara, Romania.

**Introduction.** In children with Down syndrome obstructive sleep apnea (OSA) is common. Predisposition to OSA is dependent on oropharyngeal anatomical peculiarities of Down syndrome and obesity is an aggravating factor. Sleep fragmentation, episodic nocturnal hypoxemia and hypercapnia result in reduced release of GH (growth hormone) during sleep and onset of the short stature. The association of hypothyroidism emphasizes the cognitive deficit due to trisomy 21 and obstructive sleep apnea. Compromising somatic growth in height is a powerful long-term consequence in children with OSA.

**Material and method.** We present the case of a 10 year and 5 months old boy with Down syndrome in which the periodic clinical and laboratory assessment identified the presence of hypertrophic adenoids and tonsils, sleep apnea, thyroid hypofunction and dyslipidemia.

**Results.** The patient is obese (BMI = 23kg / m<sup>2</sup> at the 95th percentile for gender and age), with residual SAO after tonsils and adenoids ablation, hypercholesterolemia and mild subclinical hypothyroidism (TSH = 5.71 uIU/ml, FT3 = 6.82 pmol/L, FT4 = 14. 27 pmol/L). Sleep polygraphy revealed mixed apnea, predominantly obstructive, with apnea-hypopnea index = 18.3/hour, average SaO<sub>2</sub> = 95%, desaturation index = 20.5/hour. It was recommended hypocaloric diet, lateral decubitus posture during sleep, substitution with potassium iodide and reevaluation in order to initiate CPAP.

**Conclusions.** Evaluation of sleep apnea and thyroid function in patients with Down’s disease is mandatory. Obstructive sleep apnea, obesity and hypothyroidism require interdisciplinary and individualized management in these patients.

## E-P08.15

### Molecular and phenotypic characterization of intronic SNP

#### rs2239809 of facioGenital dysplasia 1 (FGD1) gene

**Y. M. Khimsuriya<sup>1</sup>, N. M. Kharod<sup>2</sup>, G. U. Padman<sup>3</sup>, J. B. Chauhan<sup>1</sup>, N. Roy<sup>1</sup>;**  
<sup>1</sup>Ashok & Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), Anand, India, <sup>2</sup>H.M. Patel Center for Medical Care and Education, Karamsad, India, <sup>3</sup>Bachpan Children Hospital and Neonatal Care, Surat, India.

**Introduction:** FacioGenital dysplasia gene (Xp11.22) mutations are associated with genetically heterozygous Aarskog-Scott syndrome (ASS) as well as Non-syndromic X-linked intellectual disability. FGD1 protein is an important regulator of events that control extracellular matrix remodeling, bone development, cell migration and also involved in the regulation of few secretory proteins. Recent studies suggest that non-coding variants are equally important for disease progression. Current study tests manifestations of intronic variant SNP rs2239809 of FGD1 in intellectually disabled children.

**Materials and Methods:** The prediction of associated intronic variants of FGD1 gene was performed using GWAS (Genome Wide Association Study)

central database and GWAS 3D web server. PCR-RFLP was performed for screening of selected intronic variants. Sanger sequencing was used to validate single nucleotide change in FGD1 gene. Clinical features were studied and compared; partial pedigree was recorded for selected patients.

**Results:** The significant alteration was found in two affected children from

different family harboring intronic variant c.659+27T>C (rs2239809) at

intron 3 of FGD1 gene. Surprisingly, female and male showed different pheno-

typic appearance for same variation. Atypical X-linked inheritance pattern

was observed in case of female patient.

**Conclusion:** Present study provides significant insight that intronic variant also affects gene functions. Our data do not support that X linked inheritance favoring FGD1 gene mutations in the affected male and female patients. Further study needed for confirmation of inheritance pattern, several pathophysiological manifestations and variability of X inactivation in case of female patients.

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## E-P08.16

### Patient with language impairment, intellectual disability, autism and de novo truncating FOXP1 variant helps to define emerging somatic phenotype associated with FOXP1 defects

**D. Prchalová<sup>1</sup>, M. Havlovicová<sup>1</sup>, M. Hančárová<sup>1</sup>, V. Stránecký<sup>2</sup>, Z. Sedláček<sup>1</sup>;**

<sup>1</sup>Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic, <sup>2</sup>Institute of Inherited Metabolic Disorders, Charles University 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic.

FOXP1 (forkhead box protein P1) is a transcription factor important in neurodevelopment. Thirteen patients with *de novo* protein-disrupting intragenic FOXP1 variants have been reported. They have language impairment, developmental delay/intellectual disability (ID) and autistic features. We report an 8-year-old boy with severe language impairment, moderate ID and atypical autism. His speech regressed at 18 months of age and started to improve again since 5 years of age. However, expressive speech is severely affected and shows no progress. The boy is hyperactive and anxious. Other features include hypotonia, mild facial dysmorphism, hypermetropia and strabismus.

Karyotyping and microarray analysis yielded no findings. Targeted next-generation sequencing using the TruSight Autism panel (Illumina) identified a private heterozygous nonsense FOXP1 mutation (NM\_032682.5:c.1319C>G, p. S440\*), which was confirmed using Sanger sequencing in the patient but in neither of his parents indicating its *de novo* nature. The aberrant transcript is likely removed by nonsense mediated decay (NMD), and FOXP1 haploinsufficiency was proposed as the main pathogenic mechanism. If the transcript escaped NMD, the p.S440\* mutation would truncate FOXP1 between the leucine zipper dimerization domain and the FOX DNA-binding domain. A recently functionally characterized neighbouring FOXP1 variant p.Y439\* showed aberrant protein localization.

Our patient supports the role of FOXP1 in language, cognition and behaviour, and helps to define the emerging characteristic somatic phenotype of FOXP1 mutation carriers which may include prominent forehead, frontal hair up-weep, arched eyebrows, hypertelorism, downslanting palpebral fissures, ptosis, low-set ears, short nose with a broad tip and smooth philtrum.

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## E-P08.18

### Deletion 21q22.3 and duplication 7q35q36.3 in a Colombian patient

**F. Ruiz-Botero, H. Pachajoa;**  
Universidad Icesi, Cali, Colombia.

**Introduction:** Genetic disorders are a major cause in the etiology of intellectual disability cases; however, the analysis by conventional techniques such as cytogenetic karyotyping only allows the detection of chromosomal alterations in approximately 9.5% of them. The inclusion of new technologies such as high resolution microarray analysis have allowed the study of alte-

rations in chromosomal segments that are less than 5Mb in length; this has led to an increase in the diagnosis of these patients up to 25%.

Case report: We report the first case of an eight-year-old Colombian girl of mixed race ancestry, with clinical features that include: delayed psychomotor and language development, intellectual disability, upward slanting palpebral fissures, divergent strabismus, low-set and rotated ears, tall and broad nasal bridge, flat philtrum, bifid uvula, posterior cleft palate, increased anteroposterior diameter of the chest, congenital heart defect type interauricular communication, scoliosis and umbilical hernia. Genetic analysis was performed using comparative genomic hybridization array, which evidenced the deletion of a region of approximately 3,608Mb on chromosome 21q22.3, and a duplication of 12,326Mb on chromosome 7q35q36.3, these alterations affect approximately 112 and 186 genes, respectively.

Conclusions: To this date, this is the first report of an associated terminal deletion of 21q and 7q duplication in a patient with delayed psychomotor development and intellectual disability. It is considered that future implementation of exome and RNA sequencing techniques, and analysis of their proteomic expression in a clinical context could lead to better analysis and interpretation of the genotype-phenotype correlation in cases similar to that described.

#### E-P08.19

#### Microdeletions/ microduplications among patients with intellectual disability in the Republic of Macedonia

G. Bozhinovski<sup>1</sup>, E. Sukarova Stefanovska<sup>1</sup>, Z. Gucev<sup>2</sup>, E. Sukarova Angelovska<sup>2</sup>, N. Angelovska<sup>2</sup>, O. Jotovska<sup>3</sup>, D. Plaseska-Karanfilska<sup>1</sup>;

<sup>1</sup>Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", Skopje, Macedonia, The Former Yugoslav Republic of, <sup>2</sup>Pediatric Clinic, Medical Faculty, Skopje, Macedonia, The Former Yugoslav Republic of, <sup>3</sup>Institute for Mental Health of Children and Youth "Mladost", Skopje, Macedonia, The Former Yugoslav Republic of.

Gains or losses of genomic regions less than 5Mb, are delineated as a cause of many genetic disorders and are frequently associated with intellectual disability (ID), multiple congenital anomalies, various dysmorphic features or autism. The use of array comparative genomic hybridization (aCGH) technology in clinical practice increases the detection rate of causative genetic imbalances in approximately 15% of patients with ID of unknown cause. Due to the simplicity and flexibility of the performance multiplex ligation probe amplification (MLPA) is a method of choice for detection of known microdeletions/ microduplications in less developed countries.

We aimed to evaluate the diagnostic yield of MLPA technique for identification of microdeletion/microduplication syndromes among patients with ID. A total of 120 patients with various degrees of ID, autism and/or different dysmorphic features were included in the study. MLPA kit P245 was used in all patients, while P371-4 were used in patients with specific aberrations. MLPA revealed chromosomal imbalances in 15 out of 120 patients (12.5%). Some imbalances were associated with well-described syndromes: Wolf-Hirschorn Syndrome or 4p16.3del. (n=2), and Prader Willi/Angelman or 15q11.2-q13.1del. (n=3). Other patients harbored uncommon chromosomal imbalances: 1p36.3 deletion, 3q29 deletion, 9q22 deletion, 15q13.2-13.3 duplication, 15q24.1 deletion, 17p11.2 deletion, 17p11.2 duplication, 17q21.31 deletion and 22q11.21 duplication. Xp deletion was also detected in one patient. Four of the rare chromosomal imbalances were confirmed and more precisely determined by array CGH analysis.

In conclusion, MLPA is rapid, low-cost technique that revealed the cause of intellectual disability in 12.5% of the patients in our setting.

#### E-P08.20

#### Primary immunodeficiency disorder associated with severe intellectual disability and abnormal brain MRI findings in a patient with 3q29 microduplication syndrome - A case report

I. Streata<sup>1</sup>, S. Sosoi<sup>1</sup>, A. L. Riza<sup>1</sup>, F. Burada<sup>1</sup>, M. G. Netea<sup>2</sup>, F. Mixich<sup>1</sup>, M. Ioana<sup>1</sup>;

<sup>1</sup>University of Medicine and Pharmacy of Craiova, Human Genomics Laboratory, Craiova, Romania, <sup>2</sup>Department of Medicine and Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

Introduction: Chromosome interstitial 3q29 microduplication syndrome (OMIM 611936) has been associated with a recognizable pattern of abnormalities such as mild to moderate cognitive impairment, cerebral palsy, speech delay, autistic traits, seizures, obesity, musculoskeletal anomalies (chest-wall and fingers deformities) and common craniofacial dysmorphic features.

Materials and method: We have investigated a 34-year old woman with severe intellectual disability, abnormal brain MRI findings suggestive for fronto-temporal dementia and primary immunodeficiency characterized through persistent mucosal infections with *Candida albicans*. Results of

EEG, karyotype, cerebral fluid analyses, abdominal ultrasound and urine/plasma metabolic screens were normal.

DNA isolated and purified from peripheral venous blood was further assessed by array-CGH platform (Roche NimbleGen, Madison, WI, USA) using an Array-CHX 3x720k slide. Copy number data was analyzed with Nexus 6.1 software (Nexus BioDiscovery, El Segundo, CA).

Results: - Assessment through Array-CGH identified 1.65 Mb duplication at 3q29. The duplicated region encompasses 29 genes including several protein coding genes such as RNF168, WDR53, TFRC, PIGX, PAK2, NCBP2, PIGZ, MFI2, BDH1, FBXO45 or DLG1 and a non-coding mRNA: mir4797

Conclusions: We described a case with typical 3q29 microduplication syndrome with severe phenotype. To our knowledge, thus is the first case with primary immunodeficiency features and expands the heterogeneous clinical spectrum of this syndrome. Consideration should be given to the above mentioned locus (3q29) in patients with recurrent fungal infections, intellectual disability and/or abnormal brain MRI findings.

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#### E-P08.21

#### Screening for copy number variations on the X chromosome in patients with unexplained intellectual disability

P. P. Henrique<sup>1,2</sup>, C. R. Lincoln-de-Carvalho<sup>1,2</sup>, M. P. de Mello<sup>1</sup>, A. P. Marques-de-Faria<sup>2</sup>;

<sup>1</sup>Laboratory of Human Molecular Genetics, Center of Molecular Biology and Genetic Engineering, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil,

<sup>2</sup>Department of Medical Genetics, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.

X-linked intellectual disability (XLID) is one of the most frequent genetic causes of intellectual disability (ID) occurring in 10-12% of all affected male individuals. Around 100 genes have been considered as determinant of XLID, however the role for many of them remains to be elucidated despite the knowledge on the great majority. Therefore, the aim of this study was to investigate copy number variations in XLID genes using Multiplex Ligation-dependent Probe Amplification technique in males with global developmental delay or ID of undetermined origin. A hundred and seven individuals were investigated using SALSA MLPA P106 MRX kit (MRC-Holland), which covers about 16 of the XLID genes, and alterations were confirmed by Real Time Polymerase Chain Reaction (qPCR). A normal invariant pattern was observed in 104 out of 107 individuals, and three showed variations that have been interpreted as duplications. One patient presented increased signal for HUWE1 gene, which plays a role in the control of neural differentiation. The second showed increased signals corresponding to regions of SCL6A8 and GDI1 genes; the former is included among genes involved in the creatine deficiency syndrome and the latter is involved with non-syndromic XLID. Conversely, the variation in ARX gene observed in MLPA analysis for the third patient was not confirmed in the qPCR assay. The first two individuals showed manifestations that support duplications as a cause of clinical phenotypes. These results reinforce the importance of including XLID gene analysis in the investigation of individuals with ID of undetermined origin.

#### E-P08.23

#### MECP2 duplication syndrome in a female patient with characteristic and novel clinical symptoms

K. Hadzsiev<sup>1,2</sup>, M. Czakó<sup>1,2</sup>, A. Löcsei-Fekete<sup>1</sup>, A. Szabó<sup>1,2</sup>, B. Melegi<sup>1,2</sup>,

<sup>1</sup>Department of Medical Genetics University of Pécs, Pécs, Hungary, <sup>2</sup>Szentágóthai Research Centre, Pécs, Hungary.

Background: MECP2 duplication syndrome is a rare disorder, occurs almost exclusively in males and is characterized by moderate to severe intellectual disability, weak muscle tone in infancy, feeding difficulties, poor or absent speech, refractory seizures and muscle spasticity, developmental delay or regression. Affected females have been described only in rare cases

Patient: Here we present a female patient who was born after uneventful first pregnancy at 34 week of gestation with a birth weight of 2120 g. At the first day of life the cardiologic examination revealed pulmonary valvular stenosis. At that time in her dysmorphic status brachycephaly, deep set ears with a preauricular fistula on the left side, short limbs and hepatomegaly were to be detected.

Later on severe feeding difficulties developed which improved around the 18th month of age, but she administered anti-reflux formula because of GERD. Her somatomotor development was severely delayed and extreme shyness, muscle spasticity and an excessive sweeting developed. During the first year of life she required some hospitalization because of recurrent airway infection

**Result:** We performed an aCGH and detected an Xq28 duplication with a size of 293-kb. The duplication contained the MECP2 and IRAK1 gene.

**Discussion:** The most observations on patient with Xq28 microduplication syndrome indicate that the minimal duplication region, including MECP2 and IRAK1 was sufficient to cause the typical clinical phenotype of MECP2 duplication syndrome.

**Conclusion:** In our patient with MECP2 microduplication syndrome beside the known clinical symptoms novel features were described.

#### E-P08.25

#### **Autosomal recessive primary microcephaly caused by deletion 8p23.1 in MCPH1 gene**

**M. Hejtmankova, V. Becvarova, L. Hnykova, J. Horacek, M. Trkova, D. Stejskal; GENNET, Praha 7, Czech Republic.**

**Case report:** We present a case where a mentally retarded female, whose previous diagnosis was referred to as autistic spectrum disorder, has primary microcephaly inherited as an autosomal recessive. Her birth weight was 2500g at 38 week of gestation. Diagnosis of microcephaly was confirmed early after birth. Head circumference was on 3rd percentile. She has not suffered epilepsy, but she has had mild dysmorphic features and moderate cognitive impairment. She was referred to geneticist to specify the diagnosis at the age of 8.

**Methods:** We have used SNP array (Illumina) analysis. The homozygous deletion of approximately 46 kb, encompassing the first three exons of the MCPH1 gene (microcephalin 1), was found in child. The phenotype in the heterozygous carrier mother was normal. The phenotype and genotype of her biological father was unknown.

**Conclusion:** Microcephaly may be part of many genetic malformation syndromes or may result from a variety of intrauterine factors. More specific and accurate diagnosis with molecular confirmation may provide a possibility of more focused health supervision, which could be essential for patient and genetic counselling in prenatal diagnosis of parents.

#### E-P08.27

#### **Copy number variants in a cohort of Romanian patients with neurodevelopmental disorders**

**A. Arghir<sup>1</sup>, S. Papuc<sup>1</sup>, C. Iliescu<sup>2</sup>, D. Craiu<sup>2</sup>, D. Barca<sup>2</sup>, B. Budisteanu<sup>2</sup>, I. Focsa<sup>1</sup>, M. Cristea<sup>1</sup>, I. Borcan<sup>1</sup>, M. Budisteanu<sup>1,2</sup>, A. Tutulan-Cunital<sup>1</sup>,**

<sup>1</sup>Victor Babes National Institute of Pathology, Bucharest, Romania, <sup>2</sup>“Prof. Dr. Alex Obregia” Clinical Hospital of Psychiatry, Bucharest, Romania.

Neurodevelopmental disorders (NDDs) are life-long impairing conditions, with high impact on the patient and family. Genetic and environmental factors are known contributors to human development and behavior. Thus, revealing the underlying genetic causes of these conditions is of great importance both in clinical practice and research.

We report on our experience with array-CGH (Agilent Technologies platform) in NDDs investigation. 140 patients, aged 2 months to 18 years were referred to our laboratory for genetic testing with intellectual disability and other clinical features (dysmorphism, congenital malformations, autism or other behavior problems, speech delay). Array-CGH findings were confirmed by FISH and qPCR tests, and parental studies were performed when necessary.

Pathological CNVs were detected in 30 patients, preferentially involving the following chromosomes: 4 (5 patients), 22 (5 patients), X (3 boys and one girl with XY karyotype and sex reversal due to DAX duplication), 9 and 3 (each, 3 patients).

Besides well-described syndromic regions (e.g. Wolf-Hirschorn, Cri-du-chat, Mowat-Wilson, Smith-Magenis, 22q11.2 deletion, Phelan-McDermid, MECP2 duplication syndromes), other genomic regions, rarely reported in patients with NDDs, were either deleted or duplicated in our cohort. Among these regions, 1q21.3-q22, 3p26.3, 9p24.3-24.1, 9q34.1 harbour candidate genes for NDDs and have the potential to define new clinical entities.

Our data illustrates the utility of array-CGH in the investigation of patients with intellectual disabilities and other neurodevelopmental disorders. Well-described CNVs, both molecularly and clinically, contribute to better defining new genomic regions involved in the pathogenesis of NDDs.

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#### E-P08.28

#### **Clinical and molecular aspects and genotype-phenotype correlation in Rett syndrome**

**P. Zengin Akkus, G. E. Utine, O. Akgun Dogan, P. O. Simsek Kiper, E. Z. Taskiran, G. Halliloglu, K. Boduroglu; Hacettepe University, Ankara, Turkey.**

**Introduction:** Rett syndrome is a neurodevelopmental disorder characterized by early neurological regression following a seemingly normal early

development, and is seen almost exclusively in females. Acquired fine and gross motor functions, language and communication skills are lost, with coexisting autonomic dysfunction and seizures. Rett syndrome is seen due to mutations in MECP2 gene and is a clinical diagnosis. However, molecular testing may reveal potential genotype-phenotype correlations.

**Materials and Methods:** Twenty-four patients with documented MECP2 mutations were evaluated for possible genotype-phenotype correlations. Median age of the patients was 6.5 years (2-24 years). Clinical severity was assessed using Pineda scoring system. Mutations of patients were grouped into methyl-CpG binding domain (MBD), interdomain (ID), transcriptional repression domain (TRD) and C-terminal (CT) mutations.

**Results:** Pineda scores were 5-23 (median 10.5) in whole group. Median scores were as follows in subgroups: 9 in MBD (n=7, range 5-15), 12 in ID (n=3, range 10-17), 11 in TRD (n=9, range 6-23) and 7 in CT (n=4, range 6-11). No statistically significant difference among median scores was found ( $p>0.05$ ). One 2-year-old patient had deletion encompassing exons 1-2, and had a Pineda score of 15.

**Conclusion:** In theory, phenotype-genotype correlations could be drawn for Rett syndrome, based on the observations that the encoded protein has distinct domains with specific functions and that various missense or nonsense mutations can affect all domains. Groups with larger numbers of patients may reveal presence of such correlations, which was statistically not possible in the present sample.

#### E-P08.29

#### **Detection of small SHANK3 deletions in patients with delayed speech and developmental delay using Array-CGH**

**F. Martínez-Granero<sup>1</sup>, M. Calvente<sup>1</sup>, J. C. Cigudosa<sup>1,2</sup>, J. Suela<sup>1</sup>,**

<sup>1</sup>NIMGenetics, Madrid, Spain, <sup>2</sup>Centro Nacional de Investigaciones Oncológicas, Madrid, Spain.

#### Introduction

The 22q13.3 deletion syndrome, also known as Phelan-McDermid syndrome (PMS), is a neurodevelopmental disorder with a wide range of deletion sizes (100 Kb to 9 Mb), and a great phenotypic variability. Most neurological features are thought to be caused by haploinsufficiency of SHANK3 gene, which encodes for a core scaffolding protein at the postsynaptic density of glutamatergic synapses. The present study aimed to detect deletions affecting only to the SHANK3 gene (<100 Kb) so that a better genotype-phenotype correlation can be achieved in patients with PMS.

#### Materials and Methods

Array-CGH including 15kb resolution for SHANK3 gene (KaryoNIM® Autism 180K array-CGH, designed by NIMGenetics®) has been used for analyzing patients suspected to bear PMS. Data analysis was done using hg19 genomic build and ADM-2 statistic (set as 6).

#### Results

We identified 8 individuals with 22q13.33 deletions, but only three of them were selected since they harboured pure SHANK3 deletions. Specifically, patient 1 and 2 showed a 50-60 Kb deletion affecting exons 10 to 23 and 4 to 23, respectively. On the other hand, patient 3 showed the smallest deletion, being 23 Kb sized and affecting exons 8 to 17. The three patients were diagnosed with developmental delay and/or intellectual disability. In addition, patient 1 and 3 also showed autism spectrum disorder.

#### Conclusions

These results supports haploinsufficiency of the SHANK3 gene, which encodes for a structural protein of the postsynaptic density, as a major causative factor in the neurological symptoms observed in Phelan-McDermid syndrome.

#### E-P08.30

#### **Syndrome of unknown etiology: female patient with severe growth and mental retardation, macrocephaly, pubertas tarda and dysgerminoma**

**M. Stampfer, S. Beck-Woedl, O. Riess, A. Riess;**

*Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen, Germany.*

We report on a 19-year old female; the second child of non-consanguineous German parents. She was born at term with normal height and weight but macrocephaly. Furthermore a meconium ileus, malrotation and an umbilical hernia were present at birth. A small atrial septal defect closed spontaneously. She developed epileptic spasms at 4 months of age, but was without further spasms under treatment. At the age of 8 years she developed atonic seizures with no reoccurrence under therapy. cMRI showed myelination disorder. Metabolic tests were normal. She learned to walk at the age of 6 years. A dysgerminoma was detected at age 14 and was treated with salpingo-oophorectomy and chemotherapy. The patient has hypothyroidism and is treated with L-Thyroxin. Her menstruation started with 18 years, she got

no pubic hair yet.

At age of 19 years, she is very small (17 cm < 3. centile), has a disproportionate stature and mild muscular hypotonia. Dysmorphic features include coarse face, macrocephaly, macroglossia, hypertelorism, smooth philtrum, thin upper lip vermillion, wide nasal base and ridge and downslanting palpebral fissures. She has short, broad thumbs, brachydactyly V and flat feet. She has severe mental retardation with minimal understanding and developed no expressive speech. She has a cheerful temper most of the time. The karyotype is 46,XX. There were no CNVs in Array-CGH (180k-chip Agilent). Next generation sequencing was performed for 525 genes associated with mental retardation (TruSight One Panel, Illumina). There was no causative mutation detected. The etiology of the syndrome remains unsolved.

#### E-P08.31

#### One novel mutation in ALDH3A2 segregated with Sjögren-Larsson syndrome in an Iran-based family; a case report

M. Taghdirt<sup>1</sup>, A. Kashef<sup>1</sup>, M. Fardaei<sup>2</sup>, M. Miryounesi<sup>3</sup>;

<sup>1</sup>Genetics center of welfare organization, Shiraz, Iran, Islamic Republic of, <sup>2</sup>Department of medical Genetics, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, <sup>3</sup>Genomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Sjögren-Larsson syndrome (SLS) is a rare type of congenital ichthyosis with neurologic signs and intellectual disability. Homozygous mutations in one gene ALDH3A2 are known to be responsible for this syndrome. This is a condition in which the specific membrane-bound fatty aldehyde dehydrogenase (FALDH) is deficient due to lack of normal alleles of ALDH3A2. In the absence of FALDH, toxic metabolites cause problem in various tissues, and mainly in brain and skin.

Here we report an interesting case of SLS from Iranian population which represents a novel two-base deletion in ALDH3A2 gene sequence accompanying major phenotypic signs of SLS including generalized ichthyosis, hyperkeratosis, pruritus, and intellectual disability.

Using standard DNA sequencing and targeted mutation analysis on the patient's DNA sample, we found a novel 2-bp homozygous deletion, c.1241\_1242delAT, resulting in a frame shift and protein truncation(p. His414Gln\*fs3).

Our findings would be of paramount importance in both diagnostic approach and molecular studies of SLS.

#### E-P08.32

#### SOX5-associated intellectual disability: an underdiagnosed syndrome?

K. Writzl<sup>1</sup>, A. Maver<sup>1</sup>, L. Kovacic<sup>2</sup>, B. Peterlin<sup>1</sup>;

<sup>1</sup>Clinical Institute of Medical Genetics, Ljubljana, Slovenia, <sup>2</sup>Novartis Ireland Ltd, Novartis, Vista Building, Elm Business Park, Dublin, Ireland.

Haploinsufficiency of SOX5, encoding a member of the SOX (SRY-related HMG-box) family of transcription factors, involved in the regulation of chondrogenesis and the development of the nervous system, was recently identified as the underlying cause of the 12p12.1 microdeletion syndrome. The syndrome is characterized by global developmental delay, intellectual disability, poor expressive speech, mild dysmorphic facial features, hypotonia, skeletal abnormalities, strabismus, behaviour abnormalities, and variable other anomalies. While eleven patients with likely causal deletions involving only SOX5 gene have already been reported in the literature, the causal role of SOX5 sequence variants is unclear with only one such variant being described in detail (Nesbitt et al., 2015).

We describe two patients with de novo variants in SOX5 identified by whole exome sequencing: one consensus splice-site variant and the other predicted pathogenic missense variant with evaluation of structural consequences by in silico protein modelling in progress. The patient with a splice-site variant presented with global developmental delay, hypotonia, strabismus, and scoliosis, which correlates well with the previously-described SOX5-associated phenotype. The patient with a missense variant had a more severe clinical course and presented with microcephaly, failure to thrive, seizures, and severe global developmental delay. No aggressive behaviour was noted in either patient. Both patients had hypoplastic corpus callosum.

In summary, these cases highlight the utility of exome sequencing in patients with genetically heterogeneous and non-specific disorders such as hypotonia, developmental delay and intellectual disability (ID) and suggest that patients with SOX5-related ID might be underdiagnosed.

#### E-P08.34

#### Clinical features and follow-up findings of Williams-Beuren Syndrome patients

B. G. Nur<sup>1</sup>, S. B. Karauzum<sup>2</sup>, E. Mihci<sup>1</sup>;

<sup>1</sup>Akdeniz University Medical School, Pediatric Genetic Department, Antalya, Turkey,

<sup>2</sup>Akdeniz University Medical School, Department of Medical Biology, Antalya, Turkey.

Introduction: Williams-Beuren syndrome(WBS) is characterized by distinctive facial features, growth delay, mental retardation with typical neurobehavioral profile, cardiovascular anomalies, endocrine anomalies, autoimmune pathologies, and infantile hypercalcemia. The aim of this study was to evaluate our WBS patients with clinical and follow-up findings in a Turkish cohort.

Materials and Methods: Twenty-four patients(12 girls,12 boys) (24 months-39 years) who diagnosed as WBS between 2000 and 2013. All patients had FISH analysis, after clinical assessment.

For obtaining uniform clinical data, a questionnaire was designed according to AAP guidelines and growth parameters were plotted on WBS growth charts. Follow-up findings and medical problems recorded and they underwent the following evaluations;

- Physical and neurologic examination
- Developmental assessment
- Cardiologic evaluation(electrocardiogram,echocardiogram), blood pressure
- Genitourinary evaluation(ultrasonography,renal function)
- Ophthalmologic evaluation
- Hearing assessment
- Dental evaluation

#### Statistical Analysis

Demographic variables and clinical features analyzed using descriptive statistics.

Results: The facial features of the patients, associated diseases were seen in table1,2.

The gastrointestinal system pathologies and symptoms were seen in table3.

Discussion: Diagnosis of this syndrome relies mainly on recognition of the characteristic facial features. The variability of the findings often makes clinical delineation difficult. In this study we performed a multisystem assessment with multidisciplinary approach and we describe the clinical features and medical complications in this cohort of patients. We must be careful for complications in the follow-up of WBS patients. Early diagnosis of pathologies and adequate management of these abnormalities are essential to improve the quality of life of the patients and to prevent other chronic diseases, and its complications.

#### E-P08.35

#### Hemophilia B in a Female with Intellectual Disability caused by a Deletion of Xq26.3q28 encompassing the F9 gene

R. Kersseboom<sup>1,2</sup>, A. Kattentidt<sup>2</sup>, F. de Vries<sup>3</sup>, A. Kievit<sup>2</sup>;

<sup>1</sup>Tragel Zorg, Clinge, Netherlands, <sup>2</sup>Zuidwester, Middelharnis, Netherlands, <sup>3</sup>Erasmus medical centre, Rotterdam, Netherlands.

Hemophilia B is an X-linked recessive disorder caused by mutations in the F9-gene on Xq27.1. Mainly males are affected but about 20% of female carriers have clotting factor IX activity below 0.40 IU/mL and bleeding problems. Fragile-X syndrome (FMR1-gene) and FRAXE syndrome (AFF2-gene) are well known causes of X-linked recessive intellectual disability. Simultaneous deletion of both the FMR1- and AFF2-gene in males results in severe intellectual disability. In females the phenotype is more variable. We report on a 19-year-old female with severe intellectual disability and a longstanding bleeding history that was diagnosed with mild hemophilia B after finding an 11 Mb deletion in Xq26.3q28 that included the IDS-, SOX3-, FMR1-, AFF2- and F9-genes. To the best of our knowledge, this is the first patient with an Xq26.3q28 deletion encompassing the FMR1-, AFF2- and F9-genes, thereby causing both intellectual disability and mild hemophilia B.

#### E-P08.02

#### 10q11.21q11.22 microdeletion in a boy with intellectual delay and multiple congenital anomalies

S. abbaspour<sup>1</sup>, P. Keshavarz<sup>1</sup>, S. Afzal<sup>2</sup>, Z. Naderi<sup>2</sup>, A. Kahnmoei<sup>2</sup>, F. Falahabadi<sup>2</sup>, A. Davoudi<sup>1,2</sup>;

<sup>1</sup>Guilan University of Medical Science, Rasht, Iran, Islamic Republic of, <sup>2</sup>Medical Genetic Laboratory of Dr. Keshavarz, Rasht, Iran, Islamic Republic of.

Background: 10q deletion syndrome is an uncommon chromosomal disorder. Features that often occur in people with 10q deletion include developmental delay, intellectual disability and distinctive facial features.

Case report: We report a case of microdeletion 10q in a boy with 11-year-old

from northern Iran. The patient was born full-term, with a natural child-birth, after a pregnancy without intercurrences. After birth, he had tend to grow more slowly than their peers. During childhood, raising the head end of the bed for sleeping and snore noisily when asleep. The patient, pronounced her first words at the age of 22 months. The patient speech was severely affected. So that the patient was unable to stating the obvious words. Because of his low IQ, unable to accompany normal school. During the physical exam at the age of 11, patient weight (20.8 kg) and height (124 cm) was somewhat lower than normal. Other facial features in patient included; position of hairline that placed lower than normal. Chin and lower jaw was small and slightly receding. Hand-eye coordination and fine motor skills in patient was slower than his peers. Her gross motor skills were delayed by 8 months. Investigation of patient teeth's showed that was much irregular dentition. Check the status of skeletal patient showed that has a curvature and deformation of the sole of the foot and heel.

Conclusion: It is strongly recommended, parents should have the opportunity to meet a genetic counsellor to discuss the specific recurrence risks to be undertaken in children.

## E-P09 Neurogenetic and psychiatric disorders

### E-P09.01

#### -759C/T polymorphism in HTR2C gene and atypical antipsychotic-induced weight gain among Romanian psychotic patients

N. Andreescu<sup>1</sup>, R. Gradinaru<sup>1</sup>, L. Nussbaum<sup>1</sup>, S. Farcas<sup>1</sup>, V. Dumitrescu<sup>1</sup>, M. Puiu<sup>1</sup>, A. Emandi Chirita<sup>2</sup>,  
<sup>1</sup>University of Medicine & Pharmacy Victor Babes, Timisoara, Romania, <sup>2</sup>University of Medicine & Pharmacy, Timisoara, Roumania, Timisoara, Romania.

Metabolic and endocrine side effects of atypical antipsychotics have become an increasing concern for clinicians and scientific researchers due to the fact that atypical antipsychotics are more and more prescribed to children and adolescents.

We aim to investigate whether the 5-HT2C - 759C/T polymorphism was associated with weight change and hyperinsulinemia in Romanian pediatric patients.

81 schizophrenic and bipolar patients, aged between 9 to 20 years (median age 15.74±4) were enrolled from University Hospital for Child and Adolescent Psychiatry and Neurology of Timisoara. All the patients were under treatment with atypical antipsychotics (Risperidone, Aripiprazole, Olanzapine). Body Mass Index was recorded for different time points and fasting blood samples were taken to measure insulinemia. 5-HTR2C -759C/T polymorphism identification was carried out.

From the study group, 22 patients presented the -759C/T polymorphism in 5-HT2C gene and we identified 2 lots based on their specific genotype: the first lot included patients with C/CC genotype and the second lot included patients with T/CT genotype. No significant statistical difference in changes of BMI from baseline to different endpoints was found between the 2 lots. A significant statistic difference (p=0.035) between the lots identified based on genotype was found for insulinemia after 18 months of treatment.

Taking into consideration that weight gain is a trait in which multiple genes involved interact with environmental influences, the development of a more complex algorithm should have better chance to be implemented as a clinical tool and maybe -759C/T polymorphism would find a definite role in this algorithm.

### E-P09.02

#### A new case with atypical 22q11.22q11.23 duplication in a boy with developmental delay and hyperactivity

L. Otoolea<sup>1</sup>, I. Martí<sup>2</sup>, J. S. Crettaz<sup>1</sup>, R. Muguerza<sup>1</sup>, Y. Ramírez<sup>1</sup>, R. Sáez<sup>1</sup>, O. Martínez-Mugica<sup>2</sup>,

<sup>1</sup>Genetics Department - Donostia University Hospital, San Sebastián, Spain,

<sup>2</sup>Neuropediatrics Department - Donostia University Hospital, San Sebastián, Spain.

The chromosomal region 22q11.22q11.23 has long been recognized to be susceptible to genomic rearrangement. The great majority of these recurrent CNVs are mediated by recombination between nonallelic homologous segmental duplication. The incidence is not estimated precisely because many individuals are without symptom. The phenotypic expressions are variable and there are some contradictory features. More recently, this genomic instability has been shown to extend distally to the commonly deleted/duplicated region, involving low copy repeats LCR22E-H. Despite the small number of reported cases, many of these shared clinical characteristics, including developmental delay, psychomotor retardation, hypotonia and epilepsy.

lepsy.

To better dissect the genotype-phenotype correlations, we report a 10 year old boy with cognitive disorder, hyperactivity and developmental delay. In the presence of a normal 46XY karyotype, CGHa is requested.

Analysis was performed on a GeneChip (Affymetrix®) platform with a Cytoscan array 750K. Data have been analyzed with the Chromosome Analysis Suite 2.0 software.

CGHa identified an interstitial inherited microduplication in the 22q11.22q11.23 region, flanked by LCR22E-H, of 2.1-Mb size. The carrier's father shows normal phenotypic features. Unlike the cases reported so far, our patient doesn't show epilepsy and muscle hypotonia.

The varied phenotypic expression, the incomplete penetrance and the number of familial cases observed, makes it exceedingly difficult to ascribe pathogenicity to these duplications.

Further accumulation of the patients as well as careful clinical assessment of both the diagnosed individuals and their carrier parents is required for full characterization of the condition to provide better diagnosis and counseling of the patients' families.

### E-P09.05

#### The TREM2 p.R47H variant has a smaller effect than APOE ε4 allele on Alzheimer's risk in Hungarian patient

R. Bencsik<sup>1</sup>, V. Reményi<sup>1</sup>, D. Csabai<sup>1</sup>, B. Szatmári<sup>2</sup>, Z. Grosz<sup>1</sup>, M. Molnár<sup>1</sup>,

<sup>1</sup>Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest, Hungary, <sup>2</sup>Richter Gedeon Ltd, Budapest, Hungary.

Most of the dementias are complex multifactorial disorders. Apolipoprotein-E (APOE) ε4 allele is a well-known risk factor of late-onset sporadic Alzheimer disease (AD). Low frequency coding variants in TREM2 are associated with increased Alzheimer disease (AD) risk, while loss of functions mutations of this gene result in autosomal recessive early-onset dementia. In this study we tested the role of TREM2 p.R47H and APOE-ε4 genotypes in early and late-onset AD and FTD in a Hungarian cohort.

The APOE ε4/ ε4 genotype and TREM2 p.R47H were investigated in 140 Hungarian patients with dementia (53 male 87 female mean age 65±13) and 71 controls without cognitive decline (40 male 31 female mean age 65±11) with PCR- RFPL methodology.

The TREM2 p.R47H mutation was found in heterozygous form in 3 patient, while it was absent in controls. The mutation allelic frequency in the AD cohort is: 1 %. (p=0,01 CI95% (0,002-0,018)). The APOE investigation detected the following genotypes: ε3/4 in 43, ε4/4 in 10 cases respectively. The frequency of the APOE ε4 allele is 45 % (p=0,45 CI95% (0,408-0,492)) in the demented population.

Our results confirm the association between TREM2 p.R47H and risk of AD, the observed effect on risk was substantially smaller than the effect of APOE ε4 allele in the Hungarian patients.

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### E-P09.06

#### An epilepsy syndrome: 16p13.11 microdeletion syndrome

M. Balasar<sup>1</sup>, P. Taşdemir<sup>1</sup>, H. Yavuz<sup>2</sup>,

<sup>1</sup>Necmettin Erbakan University Medical Faculty, Department of Medical Genetics, KONYA, Turkey, <sup>2</sup>Necmettin Erbakan University Medical Faculty, Department of Pediatric Neurology, KONYA, Turkey.

Introduction: Microdeletions in 16p13.11 region can cause neuropsychiatric disorders such as schizophrenia, autism, mental retardation and epilepsy. In recent years it has been shown that 16p13.11 microdeletion syndrome is a rare epilepsy syndrome. The estimated prevalence of the disease is about 1/14000. Our case was referred to the hospital with afebrile seizures when he was 7 months old. He had been suffering afebrile seizure 3-4 times a day for a month. He had no history of other diseases. Physical examination was normal. His parents were not related. His mother had a history of abortion. It has been learned that, his mother and grandfather had hearing loss and his other grandfather had schizophrenia. The case's ocular fundus examination, hearing test, heart examination and echocardiography, abdominal ultrasonography, brain magnetic resonance examination was normal. Materials and Methods: EEG, conventional cytogenetics analysis and array-CGH analysis were performed. Results: The patient was diagnosed with infantile spasms considering EEG findings and he was treated. Chromosome analysis was performed on peripheral blood and the karyotype was 46,XY. Array-CGH analysis result was arr[hg19]16p13.11p12.3(15,404,452-18,669,725) x1. 3.265 kb deletion was determined in the p arm of chromosome 16. Genetic counseling was given to family. Array-CGH analysis was also planned for his parents. Conclusions: Some deletions and/or duplications are known to cause some sporadic epilepsy syndromes in the human genome. An

16p13.11 microdeletion case have been presented here in order to emphasize the importance of the Array-CGH analysis in such cases.

#### E-P09.07

#### Autism and seizure in a patient with maternal derived 15q11.2-q13.1 duplication - Case report

**M. Martinez Atienza**<sup>1,2</sup>, **S. Garcia Linares**<sup>1,2</sup>, **A. Rubio Lopez de la Oliva**<sup>1,2</sup>, **A. Gonzalez Ramirez**<sup>3,2</sup>, **A. Mora Guijosa**<sup>1,2</sup>, **M. Perez Sanchez**<sup>1,2</sup>;

<sup>1</sup>Complejo Hospitalario de Granada. SAS., Granada., Spain, <sup>2</sup>Instituto biosanitario de Granada, Granada, Spain, <sup>3</sup>FIBAO. Complejo Hospitalario de Granada. SAS, Granada, Spain.

Proximal 15q duplications that include the critical region for Prader-Willi syndrome (PWS) and angelman syndrome (AS) have been reported in patients with autism. Of the cases of autism reported to have duplications of the PWS/AS critical region, all have been maternal in origin.

We describe here a female patient in which autism segregates with maternal inheritance of a proximal 15q duplication. The patient was born at 37 gestation weeks with a weigh of 3150 gr. and no incidences in neonatal period. In posterior pediatric revision was detected language and cognitive delayed, suspected due to autistic disorder (AD), at 3 years age preset epilepsy episodes. The familiar history include father and maternal grandmother with epilepsy, maternal uncle with autism and a sister healthy.

Comparative Genomic Hybridization (CGH-Array) with the Nimblegen CGX Cytogenetic Microarrays platform, suplied by PerkinElmer, was performed for the patient and parents

The CGH- Array was normal in the father and both mother and patient presented a duplication of 5,56 Mb in 15q11.2-q13.1 PWS/AS region (arr(hg19)15q11.2-q13.1(22,822,019-28,379,369)x3).

The Array-CGH from grandmother, maternal uncle and sister were not done. This results are according with previous studies that show the correlation between this duplication and autistic disorder when have been maternal in origin. The family history with the maternal uncle diagnosed of autistic disorder also agree this possibility. The epilepsy, presented in some members of both families, can be originated for another unknown genetics alteration, without any correlation with the PWS/AS region.

#### E-P09.08

#### Novel single base pair deletion in ATM cause ataxia telangiectasia in an Iranian proband

**A. Salehi Chaleshtori**<sup>1</sup>, **M. Ahmadvand**<sup>2</sup>, **O. Bashti**<sup>1</sup>, **M. Noruzinia**<sup>1,3</sup>;

<sup>1</sup>Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of, <sup>2</sup>Department of Hematology and Blood Banking, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of, <sup>3</sup>Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran, Islamic Republic of.

Introduction: Ataxia-telangiectasia is a rare disorder caused by mutations in ATM gene. This gene produces a serine/threonine protein kinase, an activator of the DNA damage response in the face of DNA DSBs, which phosphorylates downstream substrates integrating with DNA repair procedure. Most ATM mutations are private mutations and, there are no mutational hotspots in the ATM gene.

Materials and Methods: We characterized an 8 years old AT patient using clinical clinical features and exome sequencing methods.

Results: A homozygous single base pair deletion in exon 43 of the ATM gene (chr11:108188201delC) that results in a frameshift and premature termination of the protein 19 amino acids downstream to codon 2101 (p.Gln2101LysfsTer19: ENST00000278616) was detected.

Conclusions: This mutation led to fundamental alterations in ATM protein structure and representation of AT lastly. This family needs careful surveillance and the reported mutation is helpful in PND procedure for the next pregnancy and other managements of the family members.

This study was funded by Sarem Cell Research Center (SCRC) of Sarem Women's Hospital.

#### E-P09.09

#### Polymorphism of a potassium/chloride transporter gene SLC12A5 is associated with autistic-like traits in healthy individuals

**S. A. Zergeroglu**<sup>1</sup>, **T. Tuncali**<sup>2</sup>, **H. D. Ozguven**<sup>3</sup>, **C. Kalaycioglu**<sup>1</sup>;

<sup>1</sup>Ankara University Institute of Health Sciences, Department of Interdisciplinary Neuroscience, Ankara, Turkey, <sup>2</sup>Ankara University School of Medicine, Medical Genetics Department, Ankara, Turkey, <sup>3</sup>Ankara University School of Medicine, Department of Psychiatry, Ankara, Turkey.

Previous studies have indicated autistic-like traits to lie on a continuum with autism spectrum disorders in the general population. In a recent stu-

dy, a K-Cl cotransporter protein, KCC2 gene SLC12A5 on chromosome 20, which previously was linked to idiopathic generalized epilepsy, familial febrile seizures and schizophrenia, was reported to be associated with functionally-impairing variants in autism spectrum disorders. Here, we report a novel association of a polymorphism, rs9074, located in the 3'UTR region of SLC12A5 with autistic-like traits in a healthy population.

A total of 75 healthy volunteers aged between 18-30 participated to the study. Autistic-like traits were quantitized on the autism-spectrum quotient (AQ) scale. AQ scores measured for each individual were comprised of five subscales of social skills, attention switching, communication, attention to details and imagination categories utilizing a previously validated version of the AQ in Turkish. SNP genotyping assays were performed with specific primers and probes in an RT-PCR platform.

rs9074 genotypes were distributed as GG:48%, GA:41.3%, AA:10.7% with allele frequencies in HWE. Total AQ ( $p=0.008$ ), social skills ( $p=0.002$ ) and attention switching ( $p=0.038$ ) subscale scores were significantly higher in AA bearers compared to GG+GA genotypes (Mann-Whitney U test). AQ scores were normally distributed. A significant association between AA genotype and social skills scores ( $p=0.014$ ) in subjects  $+0.5$  SD above average for total AQ (normally distributed) were also noted (Fisher's exact test).

These results suggest a possible role of KCC2 in neurodevelopmental stages in liaison with autism spectrum disorders.

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#### E-P09.10

#### The association analysis of CNTNAP2 gene (rs2710102 and rs759178) with autism in a South African population

**Z. Arieff**<sup>1</sup>, **Z. Noordien**<sup>2</sup>, **C. Jacobs**<sup>1</sup>, **J. Sharma**<sup>1</sup>;

<sup>1</sup>University of the Western Cape, Cape Town, South Africa, <sup>2</sup>University of the Western Cape, Cape Town, South Africa.

#### The association analysis of CNTNAP2 gene (rs2710102 and rs759178) with autism in a South African population.

**Z. Arieff**<sup>1</sup>, **Noordien**<sup>2</sup>, **Jacobs**<sup>1</sup>, **CW**<sup>1</sup>, **J. R. Sharma**<sup>1</sup> <sup>1</sup>Department of Biotechnology, University of Western Cape, Private Bag X17, Bellville 7535, South Africa, South Africa,

Introduction: Autism is a highly heritable, heterogeneous, early onset neuro-developmental condition characterized by three core pervasive impairments. This condition is clinically heterogeneous with complex etiopathogenesis which can be conceptualized as a dynamic interplay between environment cues and predisposing genetic factors. Recent studies have reported that variants of the *CNTNAP2* gene are associated both with language deficits and language delays in autism. So *CNTNAP2* is considered as a strong candidate for the pathogenesis of autism spectrum disorders (ASD). Material and Methods: A case-control study was performed to identify association of two SNPs (rs2710102 and rs759178) of *CNTNAP2* gene and ASD in a South African population. Genomic DNA was extracted from oral swab samples of 117 cases and 123 controls and SNP genotypes were determined. The Taqman ®Real-Time PCR and genotyping assay were utilized to determine the genotypes. Results and Conclusion: None of the SNPs (rs2710102 and rs759178) within *CNTNAP2* tested are associated significantly with ASD. These negative findings may be due to the limited sample size in the present study.

#### E-P09.11

#### Religious visual hallucinations in behavioural variant FTD (bvFTD) with progranulin mutations: a case report.

**I. Manna**<sup>1</sup>, **A. Fratto**<sup>2</sup>, **M. Morelli**<sup>2</sup>, **P. Perrotta**<sup>3</sup>, **F. Calabria**<sup>3</sup>, **G. Cascini**<sup>4</sup>, **C. Chiriaco**<sup>3</sup>, **A. Quattrone**<sup>1,3</sup>;

<sup>1</sup>CNR-1 Institute of Molecular Bioimaging and Physiology (IBFM), Section of Germaneto, Catanzaro, Italy, <sup>2</sup>Institute of Neurology, Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy, Catanzaro, Italy, <sup>3</sup>Neuroimaging Research Unit, Institute of Molecular Bioimaging and Physiology, National Research Council, Catanzaro, Italy, Catanzaro, Italy, <sup>4</sup>Institute of Radiology, Division of Nuclear Medicine, Magna Graecia University, Catanzaro, Italy, Catanzaro, Italy.

Visual hallucination (VH) is a visual percept, experienced when awake, which is not elicited by an external stimulus. VHs are well described in several neurodegenerative disorders as dementia with Lewy body, prion diseases, Alzheimer's disease. At last, VHs are reported in Frontotemporal dementia (FTD), a genetically and pathologically heterogeneous neurodegenerative disorder. Mutations in MAPT, GRN and C9orf72 genes have been shown as the major causes of FTD. In particular, VHs are reported in about 30% of patients with behavioural-variant FTD (bvFTD) due to GRN mutations and in about 38% of cases with C9orf72 mutations. Here, we describe an unusual bvFTD clinical case of a 72-year-old woman, with autosomal dominant familial history of behavioural disorders, presented with a ten-

years clinical history of progressive mood symptoms such as anxiety, apathy, depression. After seven years from disease onset bilateral hand tremor, generalized slowness of movements and strong hyperreligiosity with visual religious hallucinations lasting for several minutes. The neuropsychological evaluation demonstrated a mild cognitive impairment more evident for executive functions. A 18F-fluorodeoxyglucose-PET showed hypometabolism in left fronto-temporal area, and 3 Tesla brain MRI demonstrated bilateral frontal and temporal lobes atrophy more predominant on left hemisphere. In this work, we reported an bvFTD patient carrying two variations in GRN gene (reference sequence NM\_002087.2): p.Cys139Arg, and c.\*78C>T. To our knowledge, this is the first report of a patient with a GRN mutation to have a peculiar bvFTD phenotype with hyper-religiosity and mystical VHs. Our data enlarge the spectrum of bvFTD phenotype related to GNR mutation.

#### E-P09.12

#### A genetics study for a Korean family with benign Rolandic epilepsy

J. Kim;

IlSan Hospital, Goyang-shi, Korea, Republic of.

Benign Rolandic epilepsy is the most common cause of epilepsy in childhood. A lot of cases show autosomal dominant pattern. Although GRIN2A gene is known to be the most frequently causal gene of benign Rolandic epilepsy, it can explain only 7.5% of familial benign Rolandic epilepsy. Other recently found can explain a few cases. We report a family without mutation in known genes of benign Rolandic epilepsy. Exome sequencing was performed in TheragenEx Bio Institute. The data was analyzed by GATK version 3.4. The results were filtered out according to mutation frequencies reported in 1000 genome, Exome Aggregation Consortium, and Korean Exome studies. The missense mutations and mutations in splicing sites were selected using SIFT, POLYPHEN2, and SPIDEX. Gene functions were predicted by DAVID. According to results of exome sequencing, there was no possible mutation in the known causal genes such as DEPDC5, PRRT2, RBFOX1, RBFOX3, ELP4, and SRPX2. Among the genes with mutations of autosomal dominant pattern, DENND4B, TMPPS7, ME1, AMIGO2, and KIZ5 genes had loss of function mutations. Among the genes which had similar function of the known causal genes, FAM189B, TMEM247, SIDT1, GRAMD1C, FAM134B, CACFD1, NPDC1, TMEM135, TMEM132C, PQLC1, and SLC52A3 genes had mutations in the coding regions. We could not find out the causal mutation of benign Rolandic epilepsy in this family. However, we found that the new unknown genes could have major role in Korean benign Rolandic epilepsy families and we could suggest 13 possible causal genes. Other studies are needed for larger Korean.

#### E-P09.14

#### CGH-arrays analysis in a series of pediatric patients suffering from neurological diseases and/or congenital malformations of unknown etiology

M. Gamundi<sup>1</sup>, B. Mañé<sup>1</sup>, E. Borràs<sup>1</sup>, I. Hernan<sup>1</sup>, B. Pascual<sup>1</sup>, R. Vidal<sup>2</sup>, V. Delgadillo<sup>2</sup>, R. Martorell<sup>3</sup>, M. Carballo<sup>1</sup>;

<sup>1</sup>Unitat de Genètica Molecular, Hospital de Terrassa, Terrassa, Spain, <sup>2</sup>Neurologia Pediátrica, Hospital de Terrassa, Terrassa, Spain, <sup>3</sup>Neonatología, Hospital de Terrassa, Terrassa, Spain.

**Background and aims:** CGH-arrays (Comparative Genomic Hybridization Arrays) are powerful and innovative technologies whose implementation is allowing an extraordinary change in the diagnosis of various diseases, especially in the diagnostic and research of intellectual disability, autism and malformation syndromes. Our aim was to analyze, by CGH-arrays, a series of patients affected by several neurological diseases and/or malformations to try to determine the etiology of the disease in these patients.

**Methods:** A total of 66 pediatric index patients with normal karyotype were analyzed for Copy Number Variations by CGH-arrays using CGX™ 8-plex (Agilent Technologies). Proband patients were clinically classified as: autism spectrum disorders (ASD) (n=30), intellectual disability (n=17), malformation syndromes (n=9), psychomotor delay (n=8), hypotonia (n=1) and early puberty (n=1).

**Results:** 10 pathogenic (or probably pathogenic) variants and 21 variants of unknown significance were detected. Pathogenic or probably pathogenic variants were identified in 3 patients with psychomotor delay (and other features), 3 patients with intellectual disability, 3 patients with ASD and in one patient with hypotonia and malformations. 6 of the 10 variants were previously described and associated to known syndromes (Prader-Willi syndrome (n=1), 1q21.1 microdeletion syndrome (n=1), 16p11.2 microdeletion syndrome (n=1), 22q11 deletion syndrome (n=2), and a tetrasomy of 15q11.2 region (n=1)).

**Discussion and conclusions:** Array CGH is a rapid and cost-effective technique for postnatal screening of unknown syndromes, intellectual disability and autism spectrum disorders. Our experience shows that a 15% of pediatric patients derived for the analysis of Copy Number Variations present a pathogenic or probably pathogenic variant.

#### E-P09.15

#### Association Studies of DRD4 and COMT Genes Polymorphisms with Fear during Childbirth in Korean Pregnant Women

M. Kim;

The Catholic University of Korea, Kyungido, Korea, Democratic People's Republic of.

**Objective:** The fear experienced during childbirth is affected by physiological, psychological and environmental factors. Dysfunctions of serotonin and dopamine neurotransmission are associated with the development of fear, pain and anxiety disorder. We examined the polymorphisms and frequency of mutations of the Dopamine receptor D4 (DRD4), Cathechol-O-methyltransferase (COMT) genes and their association with the fear that Korean pregnant women experience during childbirth.

**Methods:** In this study, 5 SNPs were studied for understanding the fear during childbirth in 534 Korean pregnant women. The relationship between the fear during childbirth and genetic polymorphism was investigated by multiple regression analysis after adjusting for parity in 293 women who gave birth to a child vaginally and who performed the Delivery Fear Scale (DFS), which was the instrument used for measuring the fear during childbirth among 534 Korean pregnant women.

**Results:** The frequencies of each SNP in the Korean pregnant women (n=534) were DRD4 -521T>C 0.47 and COMT +186C>T 0.30, +1158C>G 0.30 and +1222G>A 0.32. No polymorphisms were found in the DRD4 +2526T>G gene. There was no genetic association between the DRD4 and COMT gene polymorphisms and fear during childbirth.

**Conclusion:** The results of this study show that these genes (DRD4, COMT) are less likely have an effect on the fear during childbirth. In order to understand the fear during childbirth, further studies will have to be focused on comprehensive research of the social, psychological, environmental factors and the interaction of the connected genes.

#### E-P09.16

#### Genetic variants on the CHRNA3/CHRNA5 genes associated with smoking quantities in a Chinese population in Taiwan

C. Wu<sup>1</sup>, W. Y. Wu<sup>1</sup>, C. Li<sup>1</sup>, W. Chien<sup>1</sup>, Y. Chang<sup>1</sup>, H. Leu<sup>2</sup>, C. Lai<sup>3</sup>, K. Hsueh<sup>4</sup>, L. Chen<sup>5</sup>, I. Y. Tsai<sup>6</sup>, K. Liang<sup>1</sup>, M. Lin<sup>1</sup>;

<sup>1</sup>Institute of Public Health, National Yang-Ming University, Taipei, Taiwan, <sup>2</sup>Healthcare Center, Taipei Veterans General Hospital, Taipei, Taiwan, <sup>3</sup>Department of Family Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, <sup>4</sup>Department of Family Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, <sup>5</sup>Department of Psychiatry, Washington University, St. Louis, MO, United States, <sup>6</sup>Institute of Health Policy and Welfare, National Yang-Ming University, Taipei, Taiwan.

**Introduction:** Tobacco smoking is one of the major risk factors for a number of chronic diseases and is the leading cause of preventable death in the world. Many genetic studies have been conducted to identify genetic variants for smoking behavior. The most widely replicated region is the nicotinic acetylcholine receptor subunit genes (CHRNA5-A3-B4) on chromosome 15q25. As most of the published genetic studies of smoking behavior were primarily European Americans or of European origin, it is of tremendous interest to elucidate if these gene variants play any role in the etiologies of smoking behavior in Chinese population in Taiwan.

**Methods:** We recruited 485 current smokers, 370 ex-smokers, and 1202 non-smokers, respectively, from the Healthcare Center in a Medical Center. We selected 6 SNPs on CHRNA5 (rs680244, rs16969968, rs518425) and CHRNA3 (rs578776, rs3743078, rs1051730) for genotyping.

**Results:** For male subjects, we found that the CHRNA5 rs518425, the CHRNA3 (rs578776, rs3743078) contributed to the increase of smoking quantities by at least 4 cigarettes per day (CPD). Besides, we observed that CHRNA5 rs680244, and CHRNA3 (rs578776, rs3743078) were associated with heavy smoking among female smokers. We also found that CHRNA5 rs680244, and CHRNA3 (rs578776, rs3743078) were related to increase the quantities of smoking by 3 to 4 CPD.

**Conclusion:** Our results demonstrated that several distinct SNPs on the CHRNA3/CHRNA5 genes were associated with smoking quantities for different gender. To the best of our knowledge, this is the first report of the association between CHRNA3/CHRNA5 gene variations and smoking quantities in Chinese population.

## E-P09.17

### Double heterozygous missense variants in *CHRNA7* in a boy with autism spectrum disorder

C. Simao de Souza<sup>1</sup>, J. Thevenon<sup>2,3</sup>, Y. Duffourd<sup>3</sup>, P. Kuentz<sup>3</sup>, C. Poe<sup>3</sup>, A. Masurel-Paulet<sup>2</sup>, J. Pinoit<sup>1</sup>, L. Faivre<sup>2,3</sup>;

<sup>1</sup>Service de pédopsychiatrie, Centre Hospitalo-Universitaire Dijon-Bourgogne, Dijon, France, <sup>2</sup>Centre de Référence Maladies du développement et Syndromes Malformatifs, Centre Hospitalo-Universitaire Dijon-Bourgogne, Dijon, France, <sup>3</sup>EA4271 GAD, Université de Bourgogne Franche Comté, Dijon, France.

**Introduction:** Dosage sensitivity of the *CHRNA7* gene, which encodes the  $\alpha 7$  nicotinic acetylcholine receptor in the human brain, is known to have a major contribution to multiple neurodevelopmental disorders, including autism spectrum disorder (ASD), schizophrenia, and intellectual disability (ID). *CHRNA7* is included in the smallest region of overlap of the 15q13.3 deletions and duplications, a hotspot for Copy number variants (CNVs) due to the presence of segmental duplications, favoring non-allelic homologous recombination (NAHR) occurrence. Heterozygous or homozygous deletions are usually associated with a neurodevelopmental phenotype of high penetrance.

**Case Report:** Using exome sequencing after negative extensive genetic work-up in a 13-year-old Caucasian boy presenting with ASD, intellectual disability and developmental delay, with no facial dysmorphism or malformations, and normal growth, we identified two heterozygous missense mutations in the *CHRNA7* gene. The first one, p.Ala124Thr in exon 5 was inherited from his asymptomatic father, and the second one p.Tyr233Cys in exon 7, occurred *de novo*.

**Conclusions:** To our knowledge, this is the first report of ASD in a boy with double heterozygous missense variants in *CHRNA7*. Given the interest towards this gene in neuropsychiatric disorders, and the interest in the genetic dissection of ASDs, international data sharing will be of importance in order to determine if missense mutations could be responsible for neurodevelopmental phenotypes.

## E-P09.19

### DNA Repair Gene Variants and The Risk of Autism Spectrum Disorder

S. Dasdemir<sup>1</sup>, M. Guven<sup>2</sup>, K. C. Pekkoc<sup>1</sup>, H. Ulucan<sup>1</sup>, B. Dogangun<sup>3</sup>, E. Kirtas<sup>3</sup>, M. T. Kadak<sup>3</sup>, M. Kucur<sup>4</sup>, M. Seven<sup>1</sup>;

<sup>1</sup>Istanbul University, Cerrahpasa Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkey, <sup>2</sup>Istanbul University, Cerrahpasa Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey, <sup>3</sup>Istanbul University, Cerrahpasa Faculty of Medicine, Department of Child and Adolescent Psychiatry, Istanbul, Turkey, <sup>4</sup>Istanbul University, Cerrahpasa Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey.

**Introduction:** Autism spectrum disorder (ASD) is a complex disorder, and its extreme heterogeneity further complicates our understanding of its biology. Epidemiological evidence from family and twin studies supports a strong genetic component in ASD etiology. Oxidative stress and abnormal DNA methylation have been implicated in the pathophysiology of ASD. Brain tissues from ASD cases showed higher levels of oxidative stress biomarkers than healthy controls in postmortem analysis. Association between oxidative stress and DNA damage has been well-known. Thus, we sought to investigate a potential link between DNA repair genes and ASD and analyze the role of XPD Asp312Asn and XRCC4 G-1394T gene polymorphisms for ASD in the Turkish population.

**Material and Methods:** Genotyping was conducted by PCR-RFLP based on 100 patients and 96 unrelated healthy controls.

**Results:** We, for the first time, demonstrated a positive association between XRCC4 gene variants and ASD risk. Frequencies of XRCC4-1394 T/G+G/G genotypes were higher in patients (%34) than the controls (%18.7). The statistical analysis revealed that the individuals who had XRCC4-1394 T/G+G/G genotype had an increased risk for ASD (OR = 2.23, 95% CI = 1.10-4.55). However, no significant association was found for XPD Asp312Asn polymorphism with the risk of ASD.

**Conclusion:** Our findings suggest that XRCC4 G-1394T polymorphism might be associated with ASD pathogenesis.

## E-P09.20

### Spectrum of *de novo* mutations in early onset severe epilepsy

H. O. Heyne, J. R. Lemke;  
University of Leipzig, Leipzig, Germany.

The field of epilepsy genetics has been constantly evolving. Next Generation Sequencing techniques, such as whole exome sequencing (WES) or targeted panels significantly facilitated the identification of underlying genetic causes of these genetically heterogeneous disorders. Multiple studies have shown that *de novo* mutations play an important role in early onset severe epilepsy representing the vast majority of pathogenic causative variants in this disease. In this study we address the distribution of *de novo* mutations

in published as well as unpublished cohorts of patients with early onset severe epilepsy. We study *de novo* mutations derived from WES trios of epilepsy patients and compare them with *de novo* mutations in healthy control cohorts. With this analysis we aim to identify the most commonly affected genes and mutational spectra in early onset severe epilepsy. Further, we intend to generate confirming or refuting evidence on genes with yet uncertain or questionable association to epilepsy.

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## E-P09.21

### Identification of rare genetic variants that cause hereditary essential tremor by high-throughput DNA sequencing

G. Houle<sup>1,2</sup>, A. Accogli<sup>3</sup>, D. Spiegelman<sup>2</sup>, P. A. Dion<sup>2,4</sup>, N. Dupré<sup>5</sup>, G. A. Rouleau<sup>2,4</sup>;

<sup>1</sup>Human Genetics Department, McGill University, Montreal, QC, Canada, <sup>2</sup>Montreal Neurological Institute and Hospital, McGill University, Montreal, QC, Canada,

<sup>3</sup>Neurosurgery Department, Istituto Giannina Gaslini, Genova, Italy, <sup>4</sup>Neurology and Neurosurgery Department, McGill University, Montreal, QC, Canada, <sup>5</sup>Department of Neurological Sciences, Faculty of Medicine, Laval University, Québec, QC, Canada.

Essential tremor (ET) is one of the most common movement disorders and is characterized by a postural or motion tremor of a body part, primarily the upper limbs. Despite a strong genetic basis for an inherited predisposition to the development of ET, genetic factors are still poorly understood. Many studies have identified ET susceptibility loci, but genetic and phenotypic heterogeneity observed across individuals make the identification of definitive causative gene difficult. Only mutations the FUS gene has been identified as a highly penetrant and causative for familial ET. However, such mutations seem to explain only a small fraction of all ET cases. We hypothesize that other rare genetic variants cause or predispose to ET. To achieve this goal, we used high-throughput DNA sequencing to capture the sequence the protein-coding portion of the genome (exome) of 16 affected individuals from four large families with an apparent autosomal dominant transmission of the disease. By focusing on rare variants in a familial cohort, we hope to explain a significant portion of the missing heritability in ET, as well as to narrow our current insight on the key biochemical pathways implicated in this complex disorder. Moreover, our findings might lead to the development of new diagnostic tests, which will help physicians make a more accurate diagnosis. [CIHR Foundation Scheme# RN254517 - 332736]

## E-P09.24

### Gomez-Lopez-Hernandez syndrome is autosomal recessive genetic inheritance? A Consanguineous marriage case

M. Elmas;

Afyon Kocatepe University, Afyonkarahisar, Turkey.

Gomez-Lopez-Hernandez syndrome (cerebello-trigeminal-dermal dysplasia) is a rare condition that includes abnormalities of the cerebellum (rhombencephalosynapsis), cranial nerves (trigeminal anesthesia), and scalp (alopecia). So far, 30 patients have been reported and one except all of them were sporadic observations. Our patient second-degree relatives of patients in her parents marriage is concerned. In her pedigree there are one relative man like her. Our patients had rhombencephalosynapsis and alopecia, but none had trigeminal dysfunction. In this respect, the term cerebello-trigeminal dermal dysplasia is potentially misleading. In conclusion, only rhombencephalosynapsis and alopecia are consistently present in GLHS and are required diagnostic criteria, while trigeminal anesthesia, dysmorphic features, and ataxia are inconsistent findings. A high index of suspicion is required to diagnose GLHS, particularly as alopecia tends to be hidden by surrounding scalp hair.

The patient was the third child for his 27-year-old mother and 31-year-old father. due to neural tube defects in prenatally brother passed a strict medical supervision . All controls in the first 16 weeks of pregnancy normally evaluated . After 16 weeks, the brain abnormalities began to be noticed. 17 weeks at the border brain hydrocephalus (14.5 mm) along with a full selection of inability to enlargement of the ventricles and the corpus callosum have been reported.

This syndromes genes unknown yet. for This family's blood collected for WES analyse and examined.

## E-P09.25

### Autosomal dominant cerebral small vessel disease associated with HTRA1 gene mutation in an Italian family

L. Veneziano<sup>1</sup>, E. Mantuano<sup>2</sup>, S. Romano<sup>2</sup>, M. Spadaro<sup>1</sup>, G. Amadori<sup>1</sup>, M. Frontali<sup>1</sup>;  
<sup>1</sup>Institute of Translational Pharmacology, National Research Council, Rome, Italy,  
<sup>2</sup>Sant'Andrea Hospital, University of Rome La Sapienza, Rome, Italy.

**Introduction:** Cerebral small vessel diseases (CSVDS) are an important cause of stroke, cognitive impairment, and mood disorders among the elderly. Usually CSVDS is sporadic, but early-onset monogenic forms of CSVDS have been reported. CADASIL is one of the most common hereditary CSVDS caused by autosomal dominant NOTCH3 gene mutations, while CARASIL is a rare autosomal recessive CSVDS caused by HTRA1 gene mutations. More than 200 mutations are known in NOTCH3 gene and only 12 HTRA1 mutated CARASIL families have been reported so far.

Recently, heterozygous HTRA1 mutations have been described associated with an autosomal dominant form of CSVDS with reduced penetrance, showing clinical features differing from those of CARASIL.

**Patients and methods:** We report a family with three affected individuals, referred for stroke and cognitive impairment segregating as an autosomal dominant disorder. Mutations in NOTCH3 and HTRA1 genes have been screened by Sanger sequencing. In silico prediction tools PolyPhen and SIFT have been used to predict the pathogenicity of the variants found. Skin biopsy of one patient was examined with transmission electron microscope.

**Results:** A heterozygous HTRA1 missense mutation segregating with the disease and predicted to be deleterious has been found. The mutation affects a highly conserved aminoacid, which, if mutated, strongly reduce the HTRA1 proteolytic activity. The biopsy showed deposits of osmophilic material in the arterial wall, very similar to those characterizing CADASIL.

**Conclusions:** We confirm the dominant inheritance of some HTRA1 mutations, overlapping clinical, MRI and ultramicroscopic features of CADASIL, in absence of the typical CARASIL extra-neurological symptoms.

## E-P09.26

### Investigation of TBP gene mutations in patients with Huntington Disease phenotype

M. Gökoğlu, H. Karabulut, Ö. Türedi, S. Altuner, C. Durmaz, E. Gokpinar, A. Vicdan, N. Yürür Kutlay, T. Tuncali, A. Tükün, H. İlgin Ruhı;  
Department of Medical Genetics, Ankara University School of Medicine, Ankara, Turkey.

**Introduction:** CAG repeat expansion in HTT gene is found nearly in 99% of clinically diagnosed Huntington Disease (HD) patients. However, in our routine genetic services, we observe this mutation in only approximately two-thirds of patients suggesting a greater contribution of genetic heterogeneity. Since TBP gene repeat expansion responsible from SCA17 is the most frequent mutation found in patients with HD phenotype, we investigated TBP mutation in this patient group.

**Materials and Methods:** Archival DNA samples of 68 patients with HD phenotype without HTT expansion were included in this study. TBP gene CAG/CAA repeat sizes were investigated by polymerase chain reaction followed by fragment analysis on ABI3130 Genetic Analyzer.

**Results:** In this patient group, CAG/CAA repeat expansions of TBP gene were all found to be in normal range (below 40).

**Conclusion:** Determining genetic basis of patients with HD phenotype is clinically important for both of differential diagnosis and genetic counselling. On the other hand, due to its extremely low frequency in this group of patients, testing for TBP CAG/CAA repeat expansion in routine genetic analysis may be considered on a case-by-case basis.

## E-P09.28

### Association study of MAOB and COMT polymorphisms in Bulgarian patients with Parkinson disease

K. Mihova<sup>1</sup>, S. Mehrabian<sup>2</sup>, R. Pavlova<sup>2</sup>, S. Skelina<sup>2</sup>, Y. Zhelev<sup>2</sup>, A. Jordanova<sup>1,3</sup>, R. Kaneva<sup>1</sup>, L. Traykov<sup>2</sup>, V. Mitev<sup>1</sup>;

<sup>1</sup>Department of Medical Chemistry and Biochemistry, Molecular Medicine Center, Medical University-Sofia, Sofia, Bulgaria, <sup>2</sup>Clinic of Neurology, University Hospital „Alexandrovska”, Department of Neurology, Medical University-Sofia, Sofia, Bulgaria,

<sup>3</sup>VIB Department of Molecular Genetics, University of Antwerp, Antwerp, Belgium.

**Background:** Parkinson disease (PD) is the second most common neurodegenerative disease characterized by motor (tremor, rigidity, postural instability, brady/akinesia etc.) and non-motor features (cognitive impairment, memory, psychiatric and personality disorders) resulting by progressive degeneration of dopaminergic neurons of substantia nigra. Monoamine oxidase B (MAOB) and catechol-O-methyltransferase (COMT) genes are associated with pathogenesis of the neurocognitive impairment.

The Val158Met (rs4680, G>A) polymorphism in COMT and intron variant

c.1348-36T>C (rs1799836, T>C) in MAOB gene have been associated with various neuropsychiatric and neurodegenerative disorders. Several studies show that they are implicated in cognitive impairment and different neuropsychiatric features.

**Materials and methods:** In this study 148 patients with PD and 108 healthy controls, matched to the patients by age, gender and ethnicity (NC) were included. Different genotypes were determined using TaqMan assay (Applied Biosystems). Statistical analysis was done using Plink toolset and chi square test.

**Results and discussion:** The Val158Met and c.1348-36T>C polymorphisms did not show significant difference in allele frequency distribution between the PD and NC group. The common Val158 allele and the 158Met allele were with almost equal allele frequencies ( $P=0.26$ ,  $OR=0.82$ ). The same trend was observed with c.1348-36T>C variant - almost equal allele frequencies ( $P=0.31$ ,  $OR=0.31$ ).

**Conclusions:** Our findings showed no association of Val158Met and c.1348-36T>C with Bulgarian PD patients. However, the limited power of the sample warrants further research in enlarged cohorts.

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## E-P09.29

### Association of l-selectin gene polymorphism and Multiple Sclerosis

S. Nasiri Takami;

Azad university, Damghan, Iran, Islamic Republic of.

**Background and aims:** Multiple Sclerosis is an autoimmune disease in the central nervous system that its etiology is not clear. L-selectin is a member of the selectin family of adhesion molecules which are important in the trafficking of leucocytes into the central nervous system. The aim of this study was evaluation of association of l-selectin polymorphism and severity of MS. **Methods:** Single nucleotide polymorphism of l-selectin gene phe206leu was analyzed in 144 patients with MS and 220 age and sex matched controls by using SSCP-PCR with specific primers method.

**Results:** There were significant differences in the alleles frequency of phe206leu polymorphism between the patients and control group ( $p=0.0005$ ). Moreover, genotype frequency of the polymorphism were significantly different between the patients and healthy group ( $p<0.0001$ ). No association was found between phe206leu polymorphism and severity of MS ( $p=0.07$ ). **Conclusions:** The results concluded that the polymorphism at heterozygote position could increases risk of pathogenesis in Multiple Sclerosis. In addition, the results showed that the heterozygote frequency in MS patients with severe disability was 100%. However, in this case consideration of more MS patient's population with severe disability is required.

## E-P09.32

### Relationship Between Obsessive Compulsive Disorder (OCD) and HLA haplotypes in Turkish Childrens

Ü. H. Lüleyap<sup>1</sup>, G. Karacaoglu<sup>1</sup>, P. Pazarci<sup>1</sup>, D. Alptekin<sup>1</sup>, A. Y. Tahiroglu<sup>1</sup>, S. Çetiner<sup>1</sup>, A. Yaman<sup>1</sup>, D. Lüleyap<sup>2</sup>;

<sup>1</sup>Cukurova University, Medical Faculty, ADANA, Turkey, <sup>2</sup>Eylül University, Medical Faculty, Izmir, Turkey.

Obsessive compulsive disorder (OCD) was once thought to be extremely rare, but recent epidemiological studies have shown it to be the fourth most common psychiatric disorder in the world. There are not many studies about the relationship between OCD and HLA genes. In this study, we aimed to research the relationship between childhood onset OCD and HLA alleles. This study includes 49 children diagnosed with OCD in Department Of Psychiatry and 88 healthy children aged between 4-12 in University of Cukurova Adana/TURKEY. The DNA of the subjects are isolated from blood and HLA alleles are amplified by using PCR and are read by LABScanTM 100 equipment. Results are evaluated by using univariate analysis and multivariate logistic regression analysis. In our study, single copy of B14, DRB3.1 and DRB16 alleles are found to increase the risk of OCD, 10.04, 5.86 and 4.90 times respectively. Also, double copy of C4 allele is found to increase the risk of OCD 7.89 times. Correct classification rate is found to be 73.7% according to the multivariate logistic regression analysis performed by 4 alleles. Despite the study in a different population determined that the effects of alleles in DRB and C locus are independent from each other on Rheumatoid Arthritis, our findings related to the association between DRB and C locus, which are two important elements of autoimmunity, on OCD in Turkish population is remarkable.

**E-P09.37****Evidence of increased Dysbindin promoter methylation in substance-induced psychosis**M. Bahar<sup>1</sup>, M. Noruzinia<sup>2</sup>, N. Beyraghi<sup>3</sup>, Z. Rezaei<sup>2</sup>;<sup>1</sup>Tarbiat Modares University, Bahar Medical Laboratory, Tehran, Iran, Islamic Republic of, <sup>2</sup>Tarbiat Modares University, Tehran, Iran, Islamic Republic of, <sup>3</sup>Department of Psychiatry, Behavioral Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Dysregulation of Dystrobrevin Binding Protein 1 or Dysbindin (DTNBP1) expression has been indicated in schizophrenia and other psychotic disorders. Genotyping investigations have failed to firmly implicate particular genotypes as significant risk conferrers. Here, we aimed to study the association of promoter CGIs methylation with the incidence and sub-phenotypes of psychosis.

In our study, we recruited patients suffering from schizoaffective (SZA), psychotic bipolar I disorder (BID) or substance-induced psychosis (n=42). In order to deconstruct psychosis to sub-phenotypes we used v4.0 Basic Psychiatric Rating Scale (BPRS). Also, we measured the levels of promoter methylation using Methylation-Specific High Resolution Melt analysis (MS-HRM) and MethylLight chemistries.

Contrary to previous reports, our results failed to show association between increased DTNBP1 promoter methylation and psychotic bipolar I and schizoaffective disorders. However, compared to SZA and BID cases and normal group, substance-induced psychosis showed more than two-fold-increase of promoter CGI methylation of DTNBP1 which survived multiple comparison ( $p<0.01$ ). Moreover, these patients scored higher on BPRS in total and on certain sub-phenotypes.

Despite occasional use of other substances, methamphetamine was the main reported substance of abuse in our series of cases. Previously, methamphetamine-related psychosis has been shown to be associated with certain genotypes of dysbindin. Therefore, despite our small sample size, previous findings in this field, in addition to the significant CGI methylation increase which we observed warrant further investigation on DTNBP1 epimutations as potential biomarkers in methamphetamine induced psychosis. This study was funded by Tarbiat Modares University as a part of Dr. Massih Bahar thesis project.

**E-P09.38****Genetics of language deficits in schizophrenia and autism: an evolutionary approach**A. Benítez-Burraco<sup>1</sup>, E. Murphy<sup>2</sup>, W. Lattanzi<sup>3</sup>;<sup>1</sup>University of Huelva, Huelva, Spain, <sup>2</sup>University College London, London, United Kingdom, <sup>3</sup>Università Cattolica del Sacro Cuore, Rome, Italy.

Both autism spectrum disorders (ASD) and schizophrenia (SZ) are highly prevalent cognitive disorders, entailing language deficits. Recent advances in genome-wide technologies have provided with a long list of candidate genes for SZ and ASD, but the gap between genes, the pathophysiology of SZ and ASD, and their distinctive cognitive and linguistic profiles still remains open. Our study aims to bridge this gap through an evo-devo approach that focuses on how language evolved in the species and that construes both conditions as poles of a continuum of modes of cognition, also encompassing typically-developing cognition. To this aim, we have performed literature mining and network analysis of known candidate genes for SZ and ASD and found that they are overrepresented among the gene believed to be important for the evolution of the human faculty of language. Many of these genes are common risk factors for both conditions. Additionally, we performed in silico data mining of previously published SZ and ASD microarray datasets and found that many of these genes important for language evolution (like CNTNAP2, DLX5, FOXP1, or ROBO2) are differentially expressed in the brains of schizophrenics and autists. We will conclude that the (substantially opposite) linguistic (dys)abilities seen in SZ and ASD patients may represent abnormal (but still related) ontogenetic itineraries for the human faculty of language and that the same factors that prompted the transition from an ape-like cognition to a human-specific cognition may explain the high prevalence of SZ and ASD, but also their related cognitive profiles.

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**E-P09.45****a case of trisomy 12p syndrome**P. Tasdemir<sup>1</sup>, M. Balasar<sup>1</sup>, H. Caksen<sup>2</sup>;<sup>1</sup>Necmettin Erbakan University Meram Medical Faculty Department Of Medical Genetics, Konya, Turkey, <sup>2</sup>Necmettin Erbakan University Meram Medical Faculty Department Of Pediatric Genetics, Konya, Turkey.

Introduction: Trisomy 12p Syndrome is a very rare chromosomal abnor-

lity. So far about 10 patients with whole chromosome 12p duplication are reported. Trisomy 12p Syndrome is characterized by craniofacial abnormalities, postnatal developmental delay, hypotonia and mental retardation. Clinical and dysmorphic features of the disease are similar to Pallister-Killian syndrome's (Mosaic Tetrasomy 12p).

**Materials and Methods:** Our case was referred to the hospital because of developmental delay, mental retardation and leukomalacia. Clinical findings were round face, high frontal bossing, low set ears, hiperthelorism, epicanthus, strabismus, wide nasal bridge, short nose, long philtrum, thin upper lip and thick-everted lower lip. Audiogram, the heart and other system examinations were normal. He had been using antiepileptic drug due to epilepsy. He was born by C/S and his weight was 3100 gram. There were no significant feature in the analysis of pedigree. It was learned that all newborn metabolic disease tests were normal.

**Results:** The karyotype analysis results were evaluated as 47,XY,+mar. We proposed Array-CGH analysis. Array-CGH result was arr[hg19]12p13.3 3p11.1(230421-34756209)x3. We concluded that marker chromosome is consisted of chromosome 12's whole short arm. We diagnosed the patient as Trisomy 12p Syndrome. His parents' karyotype analyses were normal. Genetic counseling was given to family.

**Conclusions:** The case's clinical findings were similar to Pallister-Killian syndrome's (Mosaic Tetrasomy 12p). In the literature it is reported that trisomy 12p Syndrome and Pallister-Killian syndrome's facial appearance similarity is associated with the enhancement of gene dosage on 12p13.31 region. This rare disease is presented here in order to contribute to the literature.

**E-P10 Neuromuscular disorders****E-P10.02****Genetic investigations in Hungarian patients affected by amyotrophic lateral sclerosis**K. Tripolszki<sup>1</sup>, N. Nagy<sup>1</sup>, P. Klivényi<sup>2</sup>, J. Engelhardt<sup>2</sup>, M. Szell<sup>1</sup>;<sup>1</sup>University of Szeged, Department of Medical Genetics, Szeged, Hungary, <sup>2</sup>University of Szeged, Department of Neurology, Szeged, Hungary.

**Introduction:** Amyotrophic lateral sclerosis (ALS) is an untreatable neurodegenerative disease characterized by neuronal loss and degeneration of the upper and lower motor neurons. Patients generally have a pure ALS phenotype, with degeneration affecting mainly neurons of the motor cortex, brainstem, and spinal cord. Affected individuals usually die of respiratory failure within 3-5 years of disease progression. Approximately 5-10 % of patients with ALS have an inherited form of the disease, nevertheless the difference between hereditary and sporadic ALS seems to be artificial. Therefore, genetic factors play a key role in all types of ALS. Our objective was to screen the variation of 3 candidate genes in Hungarian patients with ALS.

**Materials and methods:** DNA from the patients was extracted from peripheral blood using standard protocols. Using targeted high-throughput sequencing, we screened mutations in the FUS, SETX and C9orf72 genes in 28 Hungarian patients with ALS.

**Results:** Genetic analysis revealed a putative novel mutation in exon 7 of the SETX gene at position 791 where an A to G (c.791A/G) substitution was identified, resulting in an asparagine to serine amino-acid exchange (N264S). The detected missense mutation is localized in the amino-terminal domain of the senataxin protein, which has a critical function in protein-protein interaction. In silico analyses suggested that the identified variation might be a pathogenic mutation.

**Conclusion:** Our results might contribute to the development of a Hungarian population-specific mutation panel and to the better understanding of ALS.

**E-P10.03****Whole Exome Sequencing Identifies a Novel SACSIN Mutation in an Extended Omani Family Segregating ARSACS and Epilepsy**

P. Scott, A. Al-Futaisi, N. Al-Yarubi, A. Al-Habsi, F. Al-Murshed;

Sultan Qaboos University Hospital, Muscat, Oman.

Autosomal recessive cerebellar ataxias (ARCA) are a heterogeneous group of rare neurological disorders affecting both the central and the peripheral nervous systems. Among these, autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a progressive neurological disorder that it is caused by homozygous or compound heterozygous mutations in *SACS* gene, encoding sacsin. Here, we described the first ever molecularly-confirmed diagnosis of ARSACS in the Arabian Peninsula achieved by a combination of homozygosity mapping and whole exome sequencing. In this multigenerational consanguineous Omani family, six affected family members share the phenotype of developmental delay, progressive ataxia, spasticity and demyelinating neuropathy as well as generalised tonic clonic seizures in

the proband. The variant c.13454T>C (p.Leu4485Ser) segregated with the disease and all assessed genotypes were consistent with health status. The missense variant appears to be novel, i.e. not in dbSNP, ExAC, LOVD and ClinVar. The amino acid substitution affects an evolutionarily conserved residue and *In silico* tools predict this variant to be pathogenic. Interestingly, this Omani family also presented with epilepsy, a feature documented but rarely observed in sacsin-related ARSACS.

#### E-P10.04

#### Expansion of 45 CAG repeats in the coding region of the ATXN2 gene: spinocerebellar ataxia. Genetic counselling-phenomenon of anticipation

*S. Izquierdo-Álvarez<sup>1</sup>, M. Palacín-Larroy<sup>2</sup>, A. Rodríguez-Valle<sup>1</sup>, M. D. Miramar-Gallart<sup>1</sup>, M. J. Alcaine-Villarroya<sup>1</sup>, A. B. Lasierra-Monclús<sup>1</sup>, E. Barrio-Oller<sup>2</sup>, J. I. González-Hevia<sup>1</sup>, <sup>1</sup>Genetic/University Hospital Miguel Servet, Zaragoza, Spain, <sup>2</sup>Neurology Service. Hospital Ernest Lluch, Calatayud, Spain, <sup>3</sup>Faculty of Medicine. University of Zaragoza, Zaragoza, Spain.*

Introduction: SCA2 presents in the third or fourth decade with truncal ataxia, dysarthria, slowed saccades and less commonly ophthalmoparesis and chorea. The disease is caused by mutations in the ataxin 2 gene ATXN2 (12q23-q24.1). The normal size of the CAG repeat is 15-24; 35 repetitions or more are associated with clinical manifestations of SCA2.

Material and Methods: patient of 22 years with progressive cerebellar ataxia 4-5 years more sensory polyneuropathy evolution. Mother with severe cerebellar involvement and behavioral disorders. In addition, a grandmother with neurological disease of unknown origin of later onset. Possible unstable CAG triplet expansions located in said region encoding genes associated with the development of spinocerebellar ataxia autosomal dominant inheritance were determined by Triplet-repeated Primed PCR (Genetic study: Labgenetics S.L. Madrid)

Results: The patient had a pathological expansion of 45 CAG repeats in the coding region of the ATXN2 gene. There is an inverse relationship between the number of CAG repeats and the age at which the disease. Appropriate genetic counseling was performed and her mother was studied with 53 years (41 CAG repeats; mutated allele with complete penetrance). Cerebellar ataxia has an autosomal dominant inheritance, in which patients with this disease usually have an affected parent.

Conclusions: In SCA2 a genetic phenomenon called anticipation occurs because the offspring of a patient affected with this disease usually have a higher number of repetitions than parents, so that the onset of the disease usually goes ahead and have a more severe phenotype, especially if the parent is male.

#### E-P10.07

#### New deletion c.2265 delT in CLCN1 gene in a case of Becker Myotonia

*A. Lafuente, M. Ortiz, F. González, G. Pi, A. Zuñiga; Hospital De La Ribera, Alzira (Valencia), Spain.*

Clinical myotonia impairs muscle relaxation after voluntary intense contraction. Myotonia congenita (MC) is an inherited myotonia due to mutations in the CLCN1 gene encoding the skeletal muscle CIC-1 chloride channel. Loss-of-function mutations of CIC-1 channel reduce the sarcolemmal chloride conductance, which, in turn, increases sarcolemma excitability and causes a delayed relaxation manifesting as a clinical and electrical myotonia. Both dominant and recessive inheritance patterns are found in MC families. Becker myotonia congenita, the recessive form, is typically more severe and has an earlier onset than the dominant one, Thomsen myotonia congenita (TMC). TMC often has a wider range of presentations, including subclinical to moderately severe forms. Consequently, these two entities may be distinguished by inheritance pattern, age at onset, and phenotype.

Proband was a male of 6 yo and he was born from non-consanguineous parents. His family history was negative for neuromuscular disorders, only his father referred muscle cramps without muscular hypertrophy. Since birth, the patient presented delayed muscle relaxation, but no painful contractures induced by cold or by emotional stress were recorded. Since 5 yo, he noticed numbness in both legs after sitting for 10-15 minutes, and walked awkwardly. He referred repeated movements can temporarily alleviate their muscle stiffness, a phenomenon known as the warm-up effect. CK levels were normal; EMG showed myotonic discharges in examined muscles. The DNA sequencing showed homozygosity for a non-described previously deletion c.2265 delT (NM.000083) in CLCN1 gene. Patient was diagnosed as Becker Myotonia because his condition was inherited in an autosomal recessive pattern.

#### E-P10.08

#### A novel PLP1 mutation in a Moroccan family with connatal Pelizaeus-Merzbacher disease

*J. Lyahyai<sup>1</sup>, B. Ouled Amar Bencheikh<sup>2,3</sup>, S. C. Elalaoui<sup>4</sup>, M. Mansouri<sup>1</sup>, L. Boualla<sup>1</sup>, A. Dionne-Laporte<sup>3,2</sup>, D. Spiegelman<sup>3,2</sup>, P. Dion<sup>3,2</sup>, P. Cossette<sup>5</sup>, G. Rouleau<sup>3,2</sup>, A. Sefiani<sup>1,4</sup>, <sup>1</sup>Centre de Génomique Humaine, Faculté de Médecine et Pharmacie de Rabat. Mohammed V University in Rabat, Rabat, Morocco, <sup>2</sup>Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada, <sup>3</sup>Montreal Neurological Institute and Hospital, McGill University, Montreal, QC, Canada, <sup>4</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, <sup>5</sup>Molecular Diagnostic Laboratory and Division of Medical Genetics, CHU Sainte-Justine, Montreal, QC, Canada.*

Introduction: Epilepsy regroups a common and diverse set of chronic neurological disorders that are characterized by spontaneous, unprovoked, and recurrent epileptic seizures. Epilepsies have a highly heterogeneous background with a strong genetic contribution and various mode of inheritance. X-linked epilepsy usually manifests as part of a syndrome or epileptic encephalopathy. The variability of clinical manifestations of X-linked epilepsy may be attributed to several factors including the causal genetic mutation, making diagnosis, genetic counseling and treatment decisions difficult. We report the description of a Moroccan family referred to our genetic department with X-linked epileptic seizures as the only initial diagnosis.

Methods: Knowing the new contribution of Next-Generation Sequencing (NGS) for clinical investigation, and given the heterogeneity of this group of disorders we performed a Whole-Exome Sequencing (WES) analysis and co-segregation study in several members of this large family.

Results: We detected a novel pathogenic PLP1 missense mutation c.251C>T (p.Ala84Asp) allowing us to make a diagnosis of Pelizaeus-Merzbacher Disease for this family.

Conclusions: This report extends the spectrum of PLP1 mutations and highlights the diagnostic utility of NGS to investigate this group of heterogeneous disorders.

#### E-P10.11

#### Diagnosis of Duchenne Muscular Dystrophy using MLPA in Brazilian Patients

*A. T. Dias<sup>1,2</sup>, É. A. Zanardo<sup>1</sup>, F. A. R. Madia<sup>1</sup>, T. V. M. M. Costa<sup>1</sup>, C. Milani<sup>1</sup>, J. G. Damasceno<sup>1</sup>, G. M. Novo-Filho<sup>1</sup>, M. M. Montenegro<sup>1</sup>, A. M. Nascimento<sup>1</sup>, M. Rocha<sup>1,3</sup>, C. A. Kim<sup>4</sup>, M. B. R. Dutra<sup>5</sup>, F. B. Piazzon<sup>1,6</sup>, F. Kok<sup>5,6</sup>, E. Zanotelli<sup>1</sup>, L. D. Kulikowski<sup>1,3,4</sup>, <sup>1</sup>Laboratório de Citogenômica do Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, <sup>2</sup>OMIKA Consultoria em Genética Ltda, São Paulo, Brazil, <sup>3</sup>Centro de Reprodução Humana e Genética do Departamento de Saúde Coletiva da Faculdade de Medicina do ABC, Santo André, Brazil, <sup>4</sup>Unidade de Genética - Departamento de Pediatria - Instituto da Criança do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, <sup>5</sup>Departamento de Neurologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, <sup>6</sup>Mendelics Genomic Analysis, São Paulo, Brazil.*

Duchenne muscular dystrophy (DMD) is a recessive X-linked disorder mainly caused by mutations in the dystrophin gene. Seventy (70%) of cases are related to exonic deletions or duplications. Multiplex Ligation-dependent Probe Amplification (MLPA) is a fast and efficient method to evaluate exonic deletions and duplications of the dystrophin gene and to predict the reading-frame of RNA level result. We investigated 49 patients with dystrophinopathy using P034 and P035 MLPA Kits - DMD mixes 1 and 2 (MRC Holland, Netherlands). The analysis was performed using GeneMarker Software (SoftGenetics, LLC). Our results revealed 35 patients (71,43%) with exonic deletions; 5 patients (10,20%) with exonic duplications and 9 patients (18,37%) with normal exonic copy number in the Dystrophin gene. Using Leiden Open Variation Database (LOVD 3.0) we found 85,7% out-of-frame deletions, 8,6% in-frame deletions and 5,7% with uncertain prediction in the 35 patients. Five (5) patients with duplications presented 100% out-of-frame duplications. The frame of mutation is related to dystrophin production (in-frame mutation with partial protein production and out-of-frame with absent or minimal protein levels). Thus, the MLPA is the most appropriate first-tier test to be applied in investigation of patients with DMD. The accurate genotype-phenotype correlation and the comprehensive analysis of the DMD mutational spectrum is an important tool to select potential candidates for mutation-specific therapies.

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#### E-P10.12

#### A 25 years of Duchenne/Becker molecular diagnosis in Spain

*R. Núñez, L. Pascual, C. Gómez, C. Prior, H. Méndez, R. de Sancho, N. Chacón, P. Lapunzina, P. Martínez, J. Molano; Hospital La Paz, Madrid, Spain.*

Introduction: Dystrophinopathies are the most common neuromuscular disorder. The disease has an X-linked inheritance and is caused by mutations in the dystrophin gene.

The most common mutations are large deletions and duplications of the

gene.

Dystrophinopathies are divided in two different clinical phenotypes, the severe form, Duchenne muscular dystrophy (DMD) and the milder form, Becker muscular dystrophy (BMD).

The objective is to describe the dystrophin mutations found in molecular diagnostic testing of dystrophinopathies in the last 25 years in La Paz Hospital (Spain).

Materials and methods: We have reviewed patients from hospitals of all over Spain, (1990-2015) with clinical suspicion of DMD/BMD. We included 136 patients with a confirmed diagnosis by molecular techniques in our laboratory.

The molecular diagnosis was made by PCR-Multiplex and/or MLPA (SALSA-P034-P035, MRCHolland). Patients with no deletions and no duplications were sequenced by Sanger or NGS.

Results: Among the 136 patients, 133 were men including 75 DMD (76%deletions, 11%duplications and 13%point mutations) and 58 BMD (90%deletions, 7%duplications and 3%point mutations). The most frequent deletions were in hotspot regions, exons 45-55 and 2-19 (DMD=23, BMD=36) and 12 novel mutations were found.

The remaining 3 patients were symptomatic women without family history, 2 adults and 1 child, all three with deletions.

Conclusions: The diagnosis of dystrophinopathies can currently be accomplished by the new molecular techniques (MLPA and NGS) up to 95%. RNA analysis could be useful to detect defect in DMD expression and deep intronic mutations.

Dystrophinopathy has to be considered in women with muscular dystrophy symptoms.

#### E-P10.18

#### Biallelic inheritance of a missense and a novel frameshift mutation in an individual with malignant hyperthermia susceptibility

C. L. Goldsmith, D. A. Dymant;

Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.

Malignant hyperthermia susceptibility (MHS) is an autosomal dominant disorder of calcium regulation in skeletal muscle. The features of an MHS event include tachycardia, increased body temperature, muscle rigidity and rhabdomyolysis upon exposure to certain anesthetic agents. MHS is genetically heterogeneous condition, though the majority of causative mutations are found in RYR1. We report a patient who experienced an MHS-event at the age of 5 years during routine surgery. Her parents subsequently underwent muscle biopsies and caffeine-halothane contracture testing (CHCT). These results were deemed positive for both her parents. Multiple relatives in the father's family also had positive CHCT test results. We initiated genetic testing of the proband using a next generation sequencing panel including RYR1, CACNA1S and STAC3 and this revealed that she was heterozygous for a mutation in exon 63 of the RYR1 gene c.9301G>A (p.Glu3104Lys) inherited from her mother) and a variant in exon 50 c.8018delA which is predicted to result in a frameshift and premature protein termination (p.His2673Profs\*73) inherited from her father. To our knowledge this variant has not been previously reported with MHS or other RYR1-related disease (central core disease, multiminicore disease). The proband and her parents are healthy and do not have any clinical findings consistent with an underlying myopathy. No additional variants or mutations in RYR1 were identified in the father after complete gene sequencing. Given his positive CHCT, the father and his relatives may have MHS due to an as yet unidentified mutation in RYR1 or other MHS-related gene.

#### E-P10.19

#### Cd95l in multiple sclerosis patients: results from a case-control study

b. mohammad hosseini pour;

Shahid Beheshti University of Medical Sciences, tehran, Iran, Islamic Republic of.

Introduction: Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS). Elimination of autoreactive T cells by activation induced cell-death (AICD) is considered to be one of major process in MS. The aim of this investigation were to evaluate expression level of FasL in whole blood from patients with Relapsing-Remitting (RR) form of MS, and to survey the association of FasL expression with risk, EDSS and duration of the disease. Methods: We compared FasL expression in 50 RR-MS patients with 50 healthy controls by TaqMan Real time PCR technique. Albeit there was an expression decrease, no statistically significant difference was found between total RR-MS patients and controls. Results:our results showed a clear association between FasL expression of females especially older than 40 years with risk of the disease

( $p=0.04$ , 95% CI= 0.387-1.14;  $p=0.003$ , 95% CI= 0.139-3.12, respectively). Moreover, there was not a significant correlation between EDSS and duration of the disease and FasL expression. This finding make a valuable question what is the principal concept for this significant association between FasL expression and risk of RR-MS in females who are older than 40 years. In this study, we failed to draw an exact expression-phenotype correlation which may be due to limited statistical confirmation as a result of the small sample size and needs more investigation. Conclusions: These findings may possibly reflect differences in the pathogenic mechanisms associated with the failure of AICD observed in this group of MS patients.

#### E-P10.22

#### Mutation analysis of 4 spinocerebellar ataxia (SCA) types in patients from southern Turkey

P. Pazarci, H. Kasap, A. F. Koc, S. Altunbasak, M. A. Erkoc;

Cukurova University Faculty of Medicine, Adana, Turkey.

Introduction: Spinocerebellar ataxias (SCA) are complex clinical and genetically heterogeneous, mostly autosomal dominant neurodegenerative diseases. At present more than 30 hereditary SCA types were associated with different gene mutations. In this study, the frequency distribution of 4 SCA types 1, 2, 3 and 6 in Turkish population was investigated with respect to clinical features.

Materials and methods: 159 patients who received diagnosis of SCA and 42 healthy controls from Adana, Mersin, Gaziantep, Hatay and Osmaniye provinces were included in the study. DNA samples were isolated from 2 mL blood samples and the number of tri-nucleotide repeats (TNRs) for each SCA type was detected by PCR-RFLP technique and sequencing.

Results: 4 SCA types were studied. Two types, SCA1 and 3, were positive and all heterozygous for expansions. SCA1 had relatively higher frequency, 4.4%, than SCA3, 0.6%. Clinical data of patients were also evaluated to correlate with the increased TNR numbers.

Conclusions: This study being the first mutation record of SCAs in this area indicated that 5.0% of cases belonged to 2 types, SCA 1 and 3.

#### E-P10.23

#### Ullrich syndrome: Identification of novel mutations in two Mexican families

C. Sanchez-Acosta<sup>1</sup>, L. Plaza-Benumea<sup>2</sup>, L. Gonzalez-Huerta<sup>3</sup>, M. Martin de Saro<sup>4</sup>, S. Cuevas-Covarrubias<sup>5</sup>;

<sup>1</sup>Departamento de Neurologia, Hospital Materno Perinatal Monica Pretelini Saenz,

ISEM, Edomex, Mexico, <sup>2</sup>Genetics Department, Children Hospital IMIEM, Edomex,

Edomex, Mexico, <sup>3</sup>Genetica, Hospital General de Mexico, Mexico City, Mexico, <sup>4</sup>Hospital

Materno Infantil ISSEMyM, Toluca, México, Edomex, Mexico, <sup>5</sup>Genetica, Facultad de Medicina, Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.

Introduction: Ullrich syndrome (US) refers to a muscular deficiency of collagen VI due to recessive and dominant mutations in the collagen VI genes. US has a prevalence of 1-9/1,000,000 in newborns, it is clinically heterogeneous although the majority of patients harbor the classical phenotype. Clinical characteristics are neonatal muscle weakness, proximal joint contractures, hyperlaxity of the distal joints, failure to thrive, lack of independent ambulation, and severe respiratory damage; in sometimes, other systemic anomalies are also present. Mutations in collagen VI genes (COL6A1, COL6A2, and COL6A3) occur in about 40% of the patients with US; they affected the alpha chains of collagen VI. Patients with mutations in these genes also can present Bethlem myopathy. Objective: To describe two novel mutations in the COL6A1 and COL6A3 genes in two Mexican families with US. Material and Methods: Genomic DNA was analyzed through whole exome sequencing, PCR and DNA sequencing analysis in US patients, non-affected members of the family and in 100 normal controls. Results: We identified two novel mutations in COL6A1 (p.344delG) and COL6A3 (p.R1998\*) genes, respectively. These mutations were not present in non-affected members of the family and in 100 normal controls; this allows to discard a possible polymorphism. Discussion and Conclusion: Modeling proteins of the mutant genotype confirmed the deleterious effect of these molecular changes in both cases. US is as disease with a heterogeneous clinical spectrum, in this study we identified two novel mutations; this finding enriched the genomic spectrum observed in US.

## E-P11 Multiple Malformation/anomalies syndromes

### E-P11.01

#### Distal 14q trisomy - cytogenetic and clinical assessment of a family with a recurrent t(14;15)(q31.1;q26)

D. A. Ozunu<sup>1</sup>, M. Gabriela<sup>1</sup>, V. Plaiașu<sup>1</sup>, M. Stoian<sup>2,3</sup>, P. Gurban<sup>2</sup>, G. Dinulescu<sup>2</sup>, C. Diaconu<sup>4</sup>;

<sup>1</sup>Mother and Child Care Institute „Prof. Dr. Alfred Rusescu”, Bucharest, Romania,

<sup>2</sup>Personal Genetics Medical Center, Bucharest, Romania, <sup>3</sup>University of Medicine and Pharmacy „Carol Davila”, Discipline of Medical Genetics, Bucharest, Romania, <sup>4</sup>Institute of Virology „Stefan S. Nicolau”, Bucharest, Romania.

Balanced chromosome translocations in either parent increases the risk of recurrent miscarriage, unbalanced chromosome rearrangements, congenital malformations, and mental retardation in liveborn offspring. Structural chromosomal aberrations can result in genetic disease due to trisomy and/or monosomy of chromosomal segments.

Chromosome 14 is often involved in chromosome rearrangements. Individual cases of partial trisomy 14q have been previously reported in the literature, in many instances resulting from parental translocations or pericentric inversions. Cases of partial trisomy 14q with a duplication of 14q31-->qter are rare and present only minor anomalies of dysmorphic features, prominent nasal, growth retardation and moderate mental retardation.

Here, we present the case of a boy of 2y7mo that was clinically assessed with facial dysmorphism, anomalies of the members, axial hypotonia and psychomotor delay. After karyotype analysis, we determined that the patient has a partial trisomy for the distal part of the long arm of chromosome 14 (14q31-->qter), confirmed by aCGH. The chromosomal abnormalities resulted from a paternal balanced translocation involving chromosomes 14 and 15 [46,XY, t(14;15)(q31;q26)], a rearrangement also found for the brother of the proband.

The inheritance and identity of the identified translocation was ascertained by extensive familial cytogenetic, FISH and aCGH studies.

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### E-P11.02

#### 19q13.11 microdeletion with minimal overlapping region for syndrome

K. T. Abe, I. M. P. O. Rizzo, A. V. Coelho, N. Sakai Jr, D. R. Carvalho, C. E. Speck-Martins; SARAH Network of Rehabilitation Hospitals, Brasília – DF, Brazil, BRASILIA, Brazil.

The 19q13.11 microdeletion syndrome (MIM 613026) is a clinically recognizable condition that recently has been identified by using microarray for genome-wide screening. We report a 9 year-old male patient with 3,7Mb deletion on 19q13.11 including the critical and minimal overlapping region (MOR) and compare our findings with those cases reported in the literature. The present patient shares several main features with the previously reported patients with 19q13.11 microdeletion syndrome including intrauterine/postnatal growth retardation and microcephaly, facial dimorphism, speech disturbance, developmental delay, hypospadias, and ectodermal dysplasia with cutis aplasia in midline scalp. In addition, brain abnormalities and ectrodactyly in hands and feet were confirmed. First diagnostic hypothesis was ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome.

The MOR encompass four zinc finger (ZNF) genes (ZNF302, ZNF181, ZNF599, ZNF30). As these ZNF act as transcription factors in brain differentiation, the haploinsufficiency of part of ZNF cluster set may be involved in the development of cognitive functions of that syndrome. Proximal to the MOR, UBA2 belongs to a protein complex with acetylation activity and several transcription factors, hormone receptors, and signaling proteins related to brain and sexual development are regulated by this post-translational modification could contribute to the clinical characteristics observed in patients.

In conclusion, we reported an additional patient affected by 19q13 microdeletion syndrome, whose characteristics may contribute to reinforce the minimal region associated and to focus on a few genes potentially responsible for the phenotype. In addition, we suggested that suspected cases of EEC syndrome could be tested to 19q13.11 microdeletion.

### E-P11.03

#### First Bulgarian case of Sotos syndrome caused by 5q35 microdeletion

D. Avdjeva-Izavella<sup>1</sup>, S. Hadjidekova<sup>2</sup>, S. Bichev<sup>3</sup>, M. Pancheva<sup>2</sup>, H. Kathom<sup>1</sup>, B. Rukova<sup>2</sup>, D. Toncheva<sup>2</sup>, R. Tincheva<sup>1</sup>;

<sup>1</sup>Department of Clinical Genetics, University Pediatrics Hospital, Medical University, Sofia, Bulgaria, <sup>2</sup>Department of Medical Genetics, Medical University, Sofia, Bulgaria,

<sup>3</sup>National Genetic Laboratory, University Hospital “Majcín Dom”, Sofia, Bulgaria.

**Introduction:** Sotos syndrome (SoS) is an autosomal dominant overgrowth syndrome characterised by a distinctive facial dysmorphism, macrocephaly, variable delays in cognitive and motor development, and advanced bone age. Most cases of SoS are caused by intragenic mutations of the *NSD1* gene or 5q35 microdeletions. The clinical presentation is independent of the underlying *NSD1* defect, although patients with microdeletions had less prominent overgrowth and more severe learning disability than cases with mutations.

**Materials and Methods:** The patient, a one-year-old boy, was referred to genetic evaluation because of the developmental delay. He was born at term after complicated pregnancy. Birth weight was 3900 g and length was 52 cm. Physical examination revealed: dolichocephaly, prominent forehead, hypertelorism, down-slanted palpebral fissures and elongated face. His weight, height and head circumference were in normal range. The abdominal ultrasound revealed a malrotated right kidney.

The genome profiling of the proband was carried out by oligo array CGH. Agilent ISCA, 4x44, v2.0, with 35 kbp backbone resolution were used. The slides were scanned on Agilent fluorescent scanner G2505C and analyzed by BlueFuse Multi, v 4.2. (20289) (BlueGnome, Cambridge, UK).

**Results:** The oligo array CGH for the patient revealed deletion of 5q35 region (5q35.2-5q35.3). The deletion was 1.66 Mb in size and encompassing *NSD1* gene. The array CGH results were confirmed by MLPA.

**Conclusions:** Point mutations in *NSD1* are common in Caucasian patients with SoS while submicroscopic deletions at 5q35 occur in approximately 10%. The present report will contribute to further understanding of genotype-phenotype correlation in Sotos syndrome.

### E-P11.04

#### 8p duplication in a newborn with hypotonia and peculiar phenotype

G. M. Varo Sánchez, E. Simarro Rueda, A. M. Gómez Pastor, M. L. Quintanilla Mata, L. Navarro Casado;

Hospital General Universitario de Albacete, Albacete, Spain.

**Introduction:** We present a newborn male patient with hypotonia and peculiar phenotype: microcephalia, microretrognathia, low hairline, hypospadias, dorsal redundant prepuce, hypermobile hips and clinodactilia. Mother's pregnancy was full-term. His birth, weight and length were normal. Parents were no relatives and they had a healthy daughter with 2 years old.

These clinical findings made the neonatologist to apply for a karyotype analysis.

Multiple birth defects are associated with an unbalanced structural chromosomal rearrangement in proportion at 5%.

**Material and methods:** Cytogenetic analysis was performed on the patient's peripheral blood. Microarray-CGH method was used for define the duplication region.

**Results:** Patient's karyotype analysis was 46,XY,dup(8)?(p11p23). Microarray CGH was performed and detected arr[hg19] 8p12p11.21 (35,930,261-41,251,797)x3. The parents karyotype analysis were normal.

**Conclusions:** The 46,XY,dup(8)?(p11p23) karyotype suggests a duplication in the region of 8p11p23. Microarray-CGH confirms the cytogenetic finding, limiting the duplication to 8p12p11.21, suggesting a causal nature.

In this case is a chromosomal abnormality de novo and it was confirmed with a normal karyotype of the parents, as the majority of the cases of duplication 8p.

Currently, the patient is 3 years old and he has an important psychomotor retardation. Besides, he shows data from autism with lack of expressive language.

### E-P11.05

#### Rare plurimaleformative syndrome marked by dermatological phenotype associated with unbalanced chromosomal anomaly

V. Plaiașu<sup>1</sup>, D. Ozunu<sup>2</sup>, G. Motel<sup>2</sup>, A. Coltoiu<sup>2</sup>, M. Stoian<sup>3</sup>, P. Gurban<sup>5</sup>, G. Dinulescu<sup>5</sup>, C. Diaconu<sup>6</sup>;

<sup>1</sup>IOMC Prof.dr.Alfred Rusescu - Genetics Department, Bucharest, Romania, <sup>2</sup>IOMC Prof. dr.Alfred Rusescu-Genetics Department, Bucharest, Romania, <sup>3</sup>IOMC Prof.dr.Alfred Rusescu-Dermatologic Department, Bucharest, Romania, <sup>4</sup>Personal Genetics-Medical Genetics Center; Discipline of Medical Genetics, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania, <sup>5</sup>Personal Genetics-Medical Genetics Center, Bucharest, Romania, <sup>6</sup>Institute of Virology “Stefan S. Nicolau”, Bucharest, Romania.

The genetic analysis of patients with multiple congenital abnormalities is a very important aid in understanding the future prognosis and development. Individually, rare chromosome disorders are extremely uncommon, with some being actually unique. Rare chromosome disorders include extra, missing or re-arranged chromosome material. Management of the condition involves early diagnosis, genetic counselling and multidisciplinary approach. We report the case of a 16-years-old female patient. She was born at term by vaginal delivery with birth weight 2100g. At the age of 12 months she showed developmental delay, she walked alone at age of 20 months and she

talks very dificile.

The patient presents a rare association of facial dysmorphism, hypopigmented phenotype with multiple lentigines, skeletal involvement with thoracic kyphosis and limited extension movements of the elbows, intellectual disability with moderate cognitive impairment. Cerebral MRI revealed corpus callosum agenesis and polymicrogyria and cardiac ultrasonography showed valvular pulmonary stenosis.

Conventional karyotype of the proband identified a constitutional derivative chromosome 9, with partial deletion of the short arm: 46,XX,del(9)(p22), confirmed by arrayCGH analysis, the deleted region containing 57 OMIM genes. The mother's constitutional chromosomal analysis was normal and the paternal karyotype was not performed because the father died by lung cancer. We cannot exclude the paternal origin of the unbalanced chromosomal anomaly.

Our patient is in agreement with the consensus phenotype of this rare condition, except skin pigmented findings. Probably some genes from the deleted region contribute to particular dermatologic feature for this syndrome. This work was supported by PN-II-PT-PCCA-2013-4-133 grant

#### E-P11.06

##### A novel splice site JAG1 mutation in a Turkish girl with Alagille Syndrome

A. Turkyilmaz, B. B. Geckinli, K. Delil, M. A. Soylemez, E. Arslan Ates, H. Simsek, M. Avsar, A. I. Guney, A. Arman, P. Ata;  
Medical Genetics Department, Marmara University Medical School, Istanbul, Turkey.

Alagille Syndrome is characterized by characteristic facial phenotype, paucity of bile ducts and cholestasis, congenital heart defects involving pulmonary stenosis, skeletal and ocular anomalies. It is caused by heterozygous mutation in the Jagged-1 gene (JAG1) on chromosome 20p12. Our patient is a 6 year-old girl who was referred to our clinic because of neonatal jaundice, failure to thrive, and pulmonary stenosis. She was born with normal physical parameters at term to unrelated parents. On her physical examination, her weight was 17 kg (10 P), length 104 cm (3 P), and head circumference 49,5 cm (10-25 P). Major clinical findings were, a broad forehead, triangular face, deep-set eyes, pointed chin, fascial xanthomas, palmar erythema, dry and excoriated skin secondary to generalized pruritus. Eye examination showed chorioretinal atrophy. Echocardiography revealed pulmonary stenosis and patent ductus arteriosus. Biochemical levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase and conjugated bilirubin were increased. In liver biopsy, multinucleated giant cell transformation of hepatocytes, periportal hepatocyte and bile duct cholestasis, mild bile ductular proliferation and portal tract neutrophilic inflammation were determined. DNA sequence analysis of JAG1 gene showed a novel de novo heterozygous donor splice-site mutation in JAG1 (c.439 +1 G>A) in intron 3 likely to disturb normal splicing. Our case has a novel mutation that hasn't been reported previously. The patients' molecular identification is important for differential diagnosis of cholestasis and genetic counseling.

#### E-P11.08

##### First the gene, then the syndrome: reverse syndromology illustrated by a child with a BCOR mutation

S. Seneca<sup>1,2</sup>, K. Stouffs<sup>1,2</sup>, A. Ghelchof<sup>1,2</sup>, M. Bonduelle<sup>1</sup>, L. De Meirlier<sup>3,2</sup>, K. Keymolen<sup>1</sup>;  
<sup>1</sup>UZ Brussels, Center for Medical Genetics, Brussels, Belgium, <sup>2</sup>Neurogenetics Research Group, Reproduction Genetics and Regenerative Medicine Research Group, Vrije Universiteit Brussel, Brussels, Belgium, <sup>3</sup>Paediatric Neurology Unit, Department of Paediatrics, UZ Brussel, Brussels, Belgium.

Introduction: Mutations in the *BCL6* Corepressor (*BCOR*) gene, located on Xp11.4, are linked to OculoFacioCardioDental syndrome (OFCD, MIM 300166). It affects both male and female patients and ophthalmological symptoms are often predominant. Materials and Methods: Exome sequencing was performed in a boy of Caucasian, non-consanguineous and healthy parents. He has one younger healthy sib. Intrauterine growth retardation was observed from 32 weeks of gestation on and he was born at 40 weeks with a weight of 2485 g and length of 46 cm. Severe pulmonary hypertension made ECMO necessary in the neonatal period. The child has delayed psychomotor development and attends a special school. At the age of 4 years, he still needs tube feeding because of severe feeding difficulties. Cardiac ultrasound shows a persistent ductus arteriosus and ophthalmological examination reveals mild microcornea. Facial gestalt is characterized by small palpebral fissures, synophrys, bilateral epicanthic folds, small ears, thin upper lip and widely-spaced small teeth. Pro- and supination are limited due to radio-ulnar synostosis. Hypospadias and cryptorchidism are present. Results and conclusions: Exome sequencing showed the presence of

a hemizygous variant, c.5000C>T, p.Ser1667Leu in the *BCOR* gene (RefSeq: NM\_001123385.1). This alteration of a highly conserved amino acid is predicted to be pathogenic (SIFT score 0.01, MutationTaster score 1, PolyPhen score 1.000). This change has not been detected in dbSNP, and ExAC shows a very low population frequency (2.3x10<sup>-5</sup>). The observed clinical features seen in this patient correspond very well with the phenotype for alterations in the *BCOR* gene as reported in the literature.

#### E-P11.09

##### Identification of a de novo novel missense mutation in FBN2 gene in a Turkish boy with Beals Syndrome

D. Kan Karaer, K. Karaer;

Department of Genetic Dr. Ersin Arslan Research and Training Hospital, Gaziantep, Turkey.

Beals syndrome is an autosomal dominantly inherited connective tissue disorder, which is also known as Beals-Hecht syndrome or Congenital contractual arachnodactyly (CCA), is classified as distal arthrogryposis syndrome Type 9 and characterized by multiple flexion contractures, arachnodactyly, severe kyphoscoliosis, abnormal pinnae and muscular hypoplasia. It is caused by a mutation in FBN2 gene on chromosome 5q3.

A newborn boy was referred to genetic counselling because of congenital anomalies and dysmorphic features. He was born to healthy non-consanguineous parents. The child had features of a long and narrow face, bilateral crumpled helix of the ears, bilateral arachnodactyly, clenched position of the hands and flexion contractures of the elbows and knees. The parents had no known history of familial birth defects. A mutation analysis of FBN2 gene was requested. The results revealed a previously unpublished sequence variant a missense c.3973G>A (p.D1325N) in a heterozygous state. In silico analysis (Mutation Taster, SIFT and PolyPhen) predicts this mutation as potentially deleterious. Sequencing of 30th exon of FBN2 gene of both parents was performed, but the variant was not found, confirming a de novo event. Functional studies and more reports are required to understand for the affect of the mutation and genotype-phenotype correlation.

#### E-P11.10

##### A new case of campomelic dysplasia with dextrocardia and without sex-reversal

I. Augustin<sup>1</sup>, O. Păvăloaia<sup>2</sup>, M. Pânzaru<sup>3</sup>, V. Martiniuc<sup>4</sup>, M. Grămescu<sup>3</sup>, L. Păduraru<sup>5</sup>, M. Stamatin<sup>5</sup>, M. Tărnavan<sup>6</sup>, M. Onofriescu<sup>6</sup>, E. Gorduza<sup>2,4</sup>,

<sup>1</sup>Student, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania, Iasi, Romania, <sup>2</sup>Medical Genetics resident, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania, Iasi, Romania, <sup>3</sup>Medical Genetics Department, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania, Iasi, Romania, <sup>4</sup>Medical Genetics Department, "Cuza-Vodă" Obstetrics and Gynaecology Hospital Iasi, Romania, Iasi, Romania, <sup>5</sup>Neonatology Department, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania, Iasi, Romania, <sup>6</sup>Obstetrics and Gynaecology Department, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania, Iasi, Romania.

We report a 3 days old male with plurimalformative syndrome, characterized by disharmonic shortstature, macrocephaly, high forehead, high anterior hairline, flat occiput, wide fontanel, low-set, posteriorly rotated ears, hypertelorism, nose with anteverted nostrils, wide and flat bridge, short, deep philtrum, microretrognathia, microstomia, cleft palate, short neck, chest hypoplasia, dextrocardia, short and curved limbs, talipes equinovarus, muscle hypotonia. The X-rays indicated reduced cranial, pelvic, tibial and fibular ossification, absence of nasal and sternal ossification, cervical kiphosis, 11 pairs of ribs, flat and short vertebral bodies, short humerus with widened distal epiphysis, shorted and curved forearm's bones and femur and hepatomegaly. Karyotype is normal. Prenatal ultrasonographic investigation of patient indicated a IUGR and short limbs. Based on these features the prenatal diagnostic supposition was achondroplasia. The diagnosis was: campomelic dysplasia (CD). CD is a rare plurimalformative syndrome (1/40.000-1/80.000 newborns) generated by mutation in SOX9 gene that codes a transcription factor expressed in embryonic stage. It controls sexual and skeletal development. Mutations are de novo and transmitted in autosomal dominant manner. In majority of 46,XY CD fetuses present a sex-reversal. Prognosis of CD is negative, 90-95% of patients died in neonatal period of respiratory insufficiency. The CD surviving patients need cardiologic, psychiatric, orthopaedic, ophthalmologic and ERT survey. The particularities of our case are absence of sexual ambiguity and presence of dextrocardia.

**E-P11.11****Is so much chromosome aberrations compatible with life?****O. Demirhan<sup>1</sup>, S. Kuleci<sup>2</sup>, & Uslu<sup>1</sup>, N. Çetinel<sup>1</sup>:**<sup>1</sup>Çukurova University, Faculty of Medicine, Dept. of Medical Biology, Adana, Turkey,<sup>2</sup>Çukurova University, Faculty of Medicine, Dept. of Chest Diseases, Adana, Turkey.

Most chromosome abnormalities (CAs) are so harmful that the embryo or fetus does not survive long enough to be born. And how much of CAs are compatible with life? We performed cytogenetic analysis by G-banding in an adult girl, aged 23 years, with hundreds of CAs, and she died after some time. While she has been evaluating for diffuse parenchymal lung disease, she was considered to have a possible genetic disorder, and asked to Department of Genetics for genetic evaluation. We observed that there were at least one or more structural and/or numerical CAs in a cell of a person. The numerical and structural aberrations were found in 94.3% of cells analyzed. However, the karyotype results were normal in 5.7% of all cells. The ratio of numerical CAs was 64.2%. These CAs were a very serious and very important genetic instability. However, these hundreds of CAs had reflected to clinic appearance with only atypical facial and growth retardation. Genomic instability is caused by DNA damage, aberrant DNA replication or uncoordinated cell division, which can lead to CAs and gene mutations. Is that so much chromosome irregularity present in a person compatible with life, and live up to a twenty-three years old?

**E-P11.14****Monosomy 2q37.2 phenotype: 18-years follow-up case report****N. Rumiantseva<sup>1</sup>, O. Khurs<sup>2</sup>:**<sup>1</sup>Republican Scientific and Practical Center, Minsk, Belarus, <sup>2</sup>Republican Scientific and Practical Center „Mother&Child“, Minsk, Belarus.

Monosomy 2q37-qter (AHO-like syndrome, OMIM 600430) shows variable manifestations depending from various deletion's size and different genes lost. Combination of moderate mental delay, overweight, brachydactyly type E (BDE) noted as recognizable pattern.

Case report. Patient's karyotype was confirmed by FISH: 46,XX,del(2)(q37.1~q37.2)dn; loss started distally from D2S2633/D2S206 (investigation was carried out by A.Polytiko, T.Liehr, Jena, Germany). Proposita (G1, P1, BW=3100g, BL=51cm, OFC=34cm) had normal neonatal history and metabolic data. In infancy girl showed hypotonia, umbilical hernia, sitting at 7 months, walked since 1 year 2 months. Toddlers period: speech delay (normal hearing), muscle weakness, wide-based gait, seizures, mild thorax deformation, hypermethrophia, astigmatism. Childhood: mental retardation, poor speech, impulsive/aggressive behaviour, reduced attention, autistic signs, cognitive deficit, overweight, vertebrae degenerative changes, short 3-4-th fingers/toes. At 18 years old: height=156cm (<10th centile), obesity (weight=80kg; >97th centile), intellectual disability, low capacity of communication, facial dysmorphisms (narrow palpebral fissures, deep-set eyes, broad nasal tip, thin upper lip), nevuses, skeletal abnormalities: short neck, Schmorl's hernias (Th11-L2), kyphosis (corset), BDE hands/feet, pes planus, hallux valgus. Normal menarche, mammary hypoplasia. US, MRI studies: normal brain, heart, hepar; abnormal gallbladder shape, retroperitoneal renal ectopia.

Conclusion. According to deletion's breakpoint the patient had haploinsufficiency of numbers genes located distally ARL4C, including candidate genes for mental/ behavior disturbances, overweight, BDE (HDAC4, PER2, GPC1, TWIST2, etc), and displayed a wide spectrum of clinical features both typical and uncommon. Patients with subtelomeric unbalance presented a longer lifespan but suffered from mental impairment/social disability and need for medical care corrected individually at each life's stage.

**E-P11.15****Case report: A novel missense mutation in BMPER gene****S. Asadollahi<sup>1,2</sup>, S. Mohammadi<sup>1</sup>, S. Seyedhassani<sup>1</sup>:**<sup>1</sup>Dr seyedhassani genetic center, Yazd, Iran, Islamic Republic of, <sup>2</sup>Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran, Islamic Republic of.

Introduction: Diaphanospondylodysostosis (DSD, MIM 608022) is a rare, recessively inherited, prenatal lethal skeletal dysplasia characterized by severe deficiency of vertebral body and sacral ossification, reduced rib number and cystic kidneys.

Case presentation: Sample was belong to an aborted fetus, which was aborted at 36 weeks of gestational age due to multiple congenital anomaly. Parents are first degree relatives and they had a previous abortion at 32 weeks of gestation due to Hydrops Fetalis. The aborted fetus had abnormal nose, low-set ear, hypoplastic lung, Polyhydramnios and Hydrops Fetalis. Nuchal translucency was reported out of range (4mm) at 12 weeks of gestation. Amniocentesis for chromosomal aneuploidy as well as fetal autopsy

was reported normal.

Material & Methods: Whole Exome Sequencing was performed using sample from proband to evaluate all exons of protein-coding genes as well as some important other genomic regions by next generation sequencing method. We identified one deleterious novel homozygous missense mutation in *BMPER* (NM\_133468) gene. Then we performed PCR and Sanger sequencing on Proband and her parents to confirm this mutation.

Results: The mutation in *BMPER* gene was in exon8:c.C664T:p.P222S. Assessment of the parents showed that they were in heterozygous state in this position. This mutation is not reported in the literature. Bioinformatics analysis by SIFT, Polyphen and Mutation Taster predicted the pathogenicity of this mutation. The CADD\_phred was 28.2.

Conclusion: Based on the above evidence, this data can be used in early diagnosis of fetal anomalies and effective genetic counseling in this family.

**E-P11.18****New deletion in EVC and EVC2 genes causing Ellis-van Creveld syndrome****E. Sarasola Díez<sup>1</sup>, S. Merino-Fernández<sup>1</sup>, M. Fernández Cuesta<sup>2</sup>, C. Ruiz Espinoza<sup>2</sup>, M. Trujillo-Tiebas<sup>3,4</sup>, V. Ruiz Pérez<sup>5</sup>, M. García-Barcina<sup>1</sup>:**<sup>1</sup>Genetics Unit - Basurto University Hospital (Osakidetza/Servicio Vasco de Salud), Bilbao, Spain, <sup>2</sup>Pediatrics Service - Basurto University Hospital (Osakidetza/Servicio Vasco de Salud), Bilbao, Spain, <sup>3</sup>Instituto de Investigación Sanitaria de la Fundación Jimenez Diaz (IIS-FJD), Hospital Universitario Jiménez Díaz, Madrid, Spain, <sup>4</sup>Centro de Investigaciones Biomédicas en Red en Enfermedades Raras (CIBERER), Madrid, Spain,<sup>5</sup>Instituto de Investigaciones Biomédicas „Alberto Sols“, IdiPAZ, UAM-CIBERER - ISCIII, Madrid, Spain.

We describe a family with three children: the first, died at birth due to cardiac complication associated to the diagnosis of Ellis-van Creveld syndrome (EvCS); the second was a healthy female, and the third one who was referred to our Genetic Counselling consultation at 31 years old. The later, showed normal intelligence, short stature (1,45cm), short ribs, maxilar hypoplasia, oligodontia and hypoplastic nails. She referred that at birth she presented interauricular communication and patent ductus arteriosus, bilateral hexadactyly and some ovoid vertebral bodies.

The EvCS and Weyers acrofacial dysostosis (WAD) are allelic disorders that differ in inheritance and clinical severity. EvCS is inherited as an autosomal recessive trait with variable expression characterized by short ribs, polydactyly, short stature and ectodermal and heart defects. Mild expression of these features, alone or in combination, have been observed in parents of patients affected of EvCS and are interpreted as manifestations of heterozygosity (so-called WAD).

In order to define the mode of inheritance in this family, the molecular study of EVC and EVC2 was performed, showing a truncating mutation in EVC gene but no other point mutation or small deletion/insertion nor in EVC neither in EVC2. This molecular finding not fully supported our clinical data. The development of a new MLPA kit (P456, MRC-Holland) allowed us to detect a heterozygous deletion encompassing part of EVC2 gene and the first exon of EVC gene, confirming the diagnosis of EvCS in our patient and, therefore, providing the adequate Genetic Counselling in this family.

**E-P11.19****De novo 15q26.2q26.3 duplication and 15q26.3 deletion in a patient with an anomalous parietal sutures****B. Aleksiniūnenė<sup>1</sup>, L. Cimbalistienė<sup>1</sup>, V. Dirsė<sup>2</sup>, E. Gineikiūnenė<sup>2</sup>, R. Marcinkutė<sup>1</sup>, A. Utkus<sup>1</sup>:**<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Hematology, Oncology and Transfusion Medicine Center at Vilnius University Hospital Santariskių Klinikos, Vilnius, Lithuania.

The combination of 15q26 duplication and deletion has not been described previously. We present a de novo 15q26.2q26.3 duplication and 15q26.3 deletion in a patient with the anomalous right parietal sutures (bipartite parietal bone) and dysmorphic facial features.

The proband is the first-born female child of non-consanguineous parents. At birth she was weighted 4042 g (90th centile) with the length of 56 cm (above 97th centile). The following dysmorphic features were noted: epicanthic folds, small nose, wide nasal bridge, opened mouth, narrow palate, small mandible and a simian crease in her left hand. At the age of two years she was diagnosed with mild developmental delay, retarded speech development and myotonia. At the age of 29 months her weight was 14700 gr (90th centile), height 100 cm (97th centile) and head circumference 49 cm (90th centile). The ultrasound examination of internal organs, including renal, was normal.

Chromosome analysis of peripheral blood lymphocytes revealed a normal karyotype 46,XX. Whole-genome SNP array was carried out and identified a de novo 15q26.2-q26.3 duplication 5.6 Mb in size and adjacent terminal

15q26.3 deletion 709 Kb in size. Detected chromosomal alterations on chromosome 15 was confirmed by real-time PCR and FISH. The duplication/deletion regions encompass over 20 genes, including NR2F2, IGF1R, MEF2A and SNRPA1 genes. IGF1R gene encodes an important component of the insulin-like growth factor axis and is critical to pre- and postnatal growth. Increased dosage of IGF1R might be responsible for the patient's phenotype and could lead to overgrowth.

#### E-P11.20

#### Interstitial deletion of chromosome 4p - case report and review of the literature

A. Mitro<sup>1</sup>, A. Apostol<sup>1</sup>, M. Aschie<sup>2</sup>, G. Cozaru<sup>1</sup>, C. Brinzan<sup>1</sup>;

<sup>1</sup>Emergency Clinical County Hospital of Constanta, Constanta, Romania, <sup>2</sup>Ovidius University of Constanta, Constanta, Romania.

To date interstitial deletion of chromosome 4p, extending from p14 from p16.1, have been described in a few cases. This proximal 4p deletion is characterized by variable degree of mental retardation, characteristic facial appearance and minor dysmorphic features.

We report a 3-year old boy with interstitial deletion in the short arm of chromosome 4p with the karyotype 46,XY,del(4)(p15.3p16.1),9qh+. For a precise delineation of the deleted region array CGH was performed. The proband's phenotype was compared with previously reported cases with similar deletion. Our case present mild mental retardation, normal growth, craniofacial dysmorphia, pectus excavatum, left undescended testes and clinodactyly of the toes. Until now our patient didn't developed epilepsy unlike other described cases and additionally has erythrocytosis with microcytosis.

#### E-P11.21

#### A Portuguese 3-generation family with KBG syndrome due to a novel mutation in ANKRD11 gene

A. M. Travessa, P. Dias, A. Medeira, A. B. Sousa;

Department of Genetics, Hospital de Santa Maria/CHLN, Lisboa, Portugal.

Introduction: KBG syndrome (OMIM 148550) is a rare disorder characterized by intellectual disability, macrodontia of the upper central incisors, typical craniofacial dysmorphism, short stature and costovertebral anomalies. KBG syndrome is caused by haploinsufficiency of the ANKRD11 gene, located at 16q24.3.

Case report: Index patient is a 42-years-old female, referred for evaluation for short stature, facial dysmorphism and mild intellectual disability. Detailed description of this patient and other affected members is provided, including photos and X-rays images. Common features to all were growth retardation/short stature, mild developmental delay/intellectual disability, typical KBG syndrome facial dysmorphism including macrodontia of the upper central incisors, and characteristic short hands with brachy-clinodactyly of the 5th finger. In the index patient's son (age 21), a cervical rib and schisis of the posterior arch of S1 were identified. Her younger daughter (age 2) had an additional history of hypotonia, congenital hearing loss and EEG with epileptic abnormalities at 15 months of age.

Methods and results: Chromosomal abnormalities were excluded in the index patient by array CGH analysis. Subsequently, a heterozygous one-nucleotide frameshift duplication c.4384dup (p.Arg1462Lysfs\*92), leading to a premature stop codon, was identified in ANKRD11 gene by Sanger sequencing, confirming KBG syndrome. Molecular confirmation of the diagnosis in the index patient's affected descendants was performed.

Conclusions: Clinical dysmorphological evaluation allowed the diagnosis of KBG and direct analysis of ANKRD11 gene revealed a novel mutation in a family with five affected patients. We aim to contribute to a better characterization of this autosomal dominant condition, highlighting its distinctive features.

#### E-P11.22

#### Kleefstra syndrome in a girl of 4 months

C. Garrido<sup>1,2</sup>, D. Escué<sup>1</sup>, M.A. Caamaño<sup>1</sup>, A. Morales<sup>1</sup>, A. Torrents<sup>1</sup>, M.M. Sánchez<sup>2</sup>, E. Geán<sup>1</sup>;

<sup>1</sup>Reference Laboratory, L'Hospitalet de Llobregat (Barcelona), Spain, <sup>2</sup>Hospital de León, León, Spain.

Introduction: Kleefstra syndrome (OMIM: 610253) is a rare genetic condition caused by a heterozygous microdeletion in about 75%, and by EHMT1 pathogenic variant in the remaining 25%. This syndrome comprises ID, childhood hypotonia and distinctive facial features. In the reviewed literature, to our knowledge only 114 cases were published so far, so the incidence is unknown in general population. The array CGH is a very powerful tool that can help us to diagnose patients with a deletion of EHMT1.

Case report: We report a case of a blood study for arrayCGH study with a wide clinical history. The clinical features of this girl of about 4 months were, psychomotor retardation with pathological motor development, axial hypotonia, lack of head control, heart disease (ASD + VSD + pulmonary stenosis), microcephaly, upper lip V-inverted-shaped, and clear skin. Consanguinity (parents cousin's brothers). Normal studies for: cytogenetic analysis, CATCH-22, cerebral ultrasound and ophthalmologic evaluation.

Results: After the processed arrayCGH by Agilent platform of 60K, we found a deletion of the genome region of the chromosome (9)(q34.4): arr[hg18] 9q34.3(138.395.514-140.138.805)x1

Conclusions: The increasing use of array CGH in clinical cytogenetic laboratories provide an efficient method to find out the causes of the pathology of the patients underdiagnosed. In familiar cases it could be a powerful tool for a good genetic counselling for future pregnancies. This must be the first test in patients with clinical features susceptible of study.

#### E-P11.23

#### Novel CEP290 mutation causing Leber congenital amaurosis in Bedouin kindred

M. Drabkin<sup>1</sup>, D. Halperin<sup>1</sup>, Y. Perez<sup>1</sup>, L. Gradstein<sup>2</sup>, O. S. Birk<sup>1,3</sup>;

<sup>1</sup>The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev, Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er Sheva, Israel, <sup>2</sup>Department of Ophthalmology, Soroka University Medical Center and Clalit Health Services, Ben Gurion University, Be'er Sheva, Israel, <sup>3</sup>Genetics Institute, Soroka University Medical Center, Ben Gurion University, Be'er Sheva, Israel.

CEP290 mutations have been previously associated with several syndromes, including Leber congenital amaurosis, Senior-Loken syndrome, Joubert syndrome, Meckel syndrome and possibly Bardet Biedl syndrome. Four individuals of large consanguineous Bedouin kindred presented with Leber congenital amaurosis with severe mental retardation. Genome-wide linkage analysis (Affymetrix 250K SNP array) identified a single 9Mbp homozygous locus in chromosome 12 unique to the affected individuals (rs2163903-rs1069742; maximum LOD score 3.6 at D12S1598). Filtering of whole exome sequencing data of an affected individual for known benign variants within this locus using open access databases (HapMap, EVS and 1000 genomes) and our in-house data of over 100 ethnically matched exomes, identified a single putatively deleterious homozygous mutation within this Locus: c.2010\_2011delA p.Ile556Phefs\*17 in CEP290. The mutation segregated as expected within the kindred and was not found in a homozygous state in further 200 ethnicity-matched controls. CEP290 encodes a centrosomal protein involved in ciliary assembly and ciliary trafficking. The phenotype caused by the novel CEP290 p.Ile556Phefs\*17 truncating mutation is of Leber congenital amaurosis. The molecular developmental mechanisms through which different syndromes evolve due to mutations affecting various positions in CEP290 are yet to be elucidated.

#### E-P11.24

#### Linear nevus sebaceous syndrome: a case report

D. Pantaleo<sup>1</sup>, M. C. Cutrupi<sup>1</sup>, M. R. Pizzino<sup>1</sup>, M. Crapanzano<sup>1</sup>, C. Pidone<sup>1</sup>, I. Loddo<sup>2</sup>, S. Briuglia<sup>1</sup>;

<sup>1</sup>Department of Paediatric Science Messina, Messina, Italy, <sup>2</sup>University of Messina, Messina, Italy.

Introduction: Linear nevus sebaceous syndrome (LNNS) is a congenital neurometabolic disorder with linear epidermal nevus. LNNS is a rare sporadic disease (<1:10.000).

Case report: we report a 10 years boy with nevi on left side of face and midline of neck. Family history was negative. He had refractory partial seizures and developmental delay. Brain MNR showed hemimegalencephaly. EEG had epileptiform bursts. There were retinal anomalies, coloboma, severe ocular hemangiomas and kyphoscoliosis. A diagnosis of LNNS was considered. Excisional biopsy of nevus confirmed diagnosis of verrucous epidermal nevus with sebaceous anomalies.

Conclusion: LNNS manifestations include linear epidermal nevus, seizures (75%), developmental delays (40%), ocular and skeletal involvement. This rare disease is hypothesized to result from postzygotic mosaic mutations in HRAS or KRAS genes. Isolated congenital sebaceous nevi are more frequent (1:1000). The risk of association with other organ involvement is greater when typical midline nevus has been identified. LNNS is a serious disease, because of cosmetic impact, malignant potential and severity of clinical manifestations. This case shows a very serious expression of LNNS, because of the organs involvement is in a very severe form. The diagnosis is difficult and late. It makes us think about the importance of early diagnosis and all children with a suspected diagnosis of LNNS should undergo a multidisciplinary follow up.

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**E-P11.25****The rare constitutional chromosomal aberration dic(X;Y) (p22.3;p11.3) in an adult male with hematological malignancy**

L. Pavlistova<sup>1</sup>, S. Izakova<sup>1</sup>, L. Bartuskova<sup>1</sup>, M. Langova<sup>2</sup>, P. Malikova<sup>3</sup>, Z. Zemanova<sup>1</sup>, K. Michalova<sup>1,4</sup>,

<sup>1</sup>Center of Oncocytogenetics, IMBLD, General Teaching Hospital and First Faculty of Medicine, Prague 2, Czech Republic, <sup>2</sup>Department of Medical Genetics, Thomayer's Hospital, Prague 4, Czech Republic, <sup>3</sup>Department of Clinical Hematology IKEM, Prague 4, Czech Republic, <sup>4</sup>Department of Cytogenetics, Institute of Hematology and Blood Transfusion, Prague 2, Czech Republic.

Constitutional translocations of gonosomes are very rare in humans and the breakpoints Xp11 and Yq11 were identified as the most frequent. Patients with breakpoints on Yp represent the not numerous subgroup of constitutional t(X;Y) giving rise to dicentric chromosome. The phenotype is variable because more factors play role: breakpoints on X and Y, presence/loss of SRY gene locus, mosaicism and X inactivation pattern.

Cytogenetic examination of bone marrow sample in 63 years old male patient with primary myelofibrosis proved the karyotype 46,X,dic(X;Y) (p22.3;p11.3)[20]. Acquired aberrations related to the myeloproliferative disorder were not identified. Beside the clone with 46,X,dic(X;Y) (p22.3;p11.3)[20], the cell line 45,X[10] was found by examination of PHA stimulated peripheral blood. FISH with probe for SHOX and SRY genes and subtelomeres of Xp/Yp (Cytocell, Abbott Molecular) proved deletion of both subtelomeric regions, SHOX gene and conserved SRY locus on dicentric chromosome. The finding was verified by mFISH (24XCyte, MetaSystems) and array CGH/SNP (BlueGnome).

Our proband have the short stature, Madelung deformity of the forearm and ear's skin abnormality. The elevation of gonadotropins and reduced level of testosterone indicate the gonadal dysfunction associated with two hereditary syndromes: heterozygous deletion of SHOX gene with Léri Weill dyschondrosteosis and additional copy of almost whole X chromosome with Klinefelter's variant syndrome.

Constitutional karyotype 46,X,dic(X;Y)(p22.3;p11.3/45,X of adult male detected in his peripheral blood and bone marrow is presented. Results of examination by clinical geneticist, cytogenetic, molecular cytogenetic, clinical and laboratory data will be discussed in detail in the poster.

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**E-P11.26****Meier-Gorlin (ear-patella-short stature) syndrome: A rare clinical entity**

Ö. Akgün Doğan<sup>1</sup>, P. Simsek Kiper<sup>1</sup>, Y. Alanay<sup>2</sup>, G. Utine<sup>1</sup>, K. Boduroğlu<sup>1</sup>,

<sup>1</sup>Hacettepe University ,Faculty of Medicine,Department of Pediatrics,Division of Pediatric Genetics, Ankara, Turkey, <sup>2</sup>Acıbadem University ,School of Medicine,Department of Pediatrics,Division of Pediatric Genetics, Istanbul, Turkey.

Meier-Gorlin (ear-patella-short stature) syndrome is a rare autosomal recessive syndrome characterized by severe prenatal and postnatal growth retardation, bilateral microtia, and absent or very small patella. The patients generally show a borderline cognitive functioning and a cheerful and friendly personality has been described by several authors. Homozygous or compound heterozygous mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* or *CDC6* have been described in the genetic etiology so far, however, in about 20% of cases, molecular etiology remains to be elucidated. We here report on clinical findings of 3 patients from 2 families presenting with facial features along with profound developmental delay. Patient 1 was born at term with a birth weight of 1750 gr (< 3<sup>rd</sup> centile) and a length of 41 cm (<3<sup>rd</sup> centile) to consanguineous parents. She was referred to our center with the complaints of developmental delay and growth retardation at the age of 1. She had dextrocardia, nephrolithiasis, and scoliosis as well. Patient 2 and Patient 3 were siblings. They were born to consanguineous parents at term with birth weights of 2700 gr (3<sup>rd</sup>-10<sup>th</sup> centiles) and 2600 gr (3<sup>rd</sup>-10<sup>th</sup> centiles), respectively. The birth lengths were not noted. They were referred to our center at the ages of 7 and 4, respectively. Both of them had facial features, developmental delay and growth retardation along with anxiety and hyperactive behavior such as fidgeting or impulsive actions. Molecular analysis of the patients presented here is still going on.

**E-P11.27****Microduplication 5q35: possible dosage effect of the NSD1 gene**

U. E. Siebers-Renelt, C. Mueller-Hofstede, P. Wieacker, A. Röpke;  
Institut für Humangenetik, Muenster, Germany.

We report on a 15-year old boy with mild mental retardation, short stature (<3<sup>rd</sup> centile), microcephaly (<3<sup>rd</sup> centile) and minor craniofacial dysmorphism. Conventional chromosome analysis revealed an apparently normal male karyotype (46,XY, GTG banding, BN 550). Array-CGH analysis (Agilent 400K microarray) uncovered a duplication of approx. 2.1Mb in the chromosomal region 5q35.2-q35.3 comprising 43 genes including the NSD1 gene. This finding was confirmed by qPCR. The interstitial character of the microduplication was proven by FISH analysis (ish 5q35.2 (RP11-627M5 enh)). Blood samples of the parents were not available.

Deletions of the NSD1 gene, located in 5q35.3, are a known cause of Sotos syndrome which belongs to the overgrowth syndromes. The Sotos critical region spans over 1.9Mb and is flanked by two low-copy repeat regions (Kurotaki et al 2005). This genomic architecture predisposes to non-allelic homologous recombination. The size and the location of the microduplication in our patient are located in the same regions suggesting a common mechanism of the generation of the genomic imbalance as already described for other chromosomal regions (e.g. 22q11.2). The main features of our patient with a microduplication 5q35 are postnatal growth retardation, microcephaly without structural brain defects and a mild to moderate mental retardation. This is in accordance to other patients with a microduplication 5q35 reported so far (Novara et al 2014). In part, the phenotype is reciprocal to Sotos syndrome suggesting a dosage effect of the NSD1 gene on growth, although the exact mechanism of NSD1 function is not known in detail yet.

**E-P11.28****A novel SF3B4 pathogenic splice variant in a 21-week-old fetus with Nager syndrome**

M. Calvello<sup>1</sup>, J. Kohlhase<sup>2</sup>, T. Rizzuti<sup>3</sup>, S. Boito<sup>4</sup>, M. F. Bedeschi<sup>1</sup>, F. Natacci<sup>1</sup>, F. Lalatta<sup>1</sup>,

<sup>1</sup>Clinical Genetics Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>2</sup>Center for Human Genetics, Freiburg, Germany, <sup>3</sup>Pathology Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy, <sup>4</sup>Obstetric Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Introduction: Nager syndrome (Acrofacial Dysostosis type 1) is characterized by craniofacial and preaxial limb anomalies. Since 2012 various loss-of-function point-mutations in SF3B4 have been identified in approximately 60% of patients with Nager syndrome. We report a 21-week-old fetus with Nager syndrome features all ascribed to a previously unreported SF3B4 mutation.

Case description: A 36-year-old woman was referred to our department at 20 weeks of gestation when morphologic ultrasound revealed severe micrognathia. A level II scan confirmed this finding and detected upper limb anomalies (short humerus, ulnar hypoplasia, undetectable radial skeleton). The couple was informed of the fetal prognosis and chose termination. Autopsy revealed severe micrognathia, low-set ears with hypoplastic antihelix, bilateral thumb agenesis, short long bones with bilateral radial aplasia and bowed ulnas, and bilateral clubfoot. Molecular analysis of the SF3B4 gene was performed on DNA extracted from amniocytes sampled before termination and from peripheral blood of both parents. A heterozygous variant c.34+1G>A in intron 1 of the SF3B4 gene was identified in fetal DNA but in neither of the parents' DNA, indicating a de novo event.

Conclusions: Since the variant detected in fetal DNA destroys the native splice donor site of intron 1, likely leading to exon skipping or to the usage of a cryptic splice site with possible nonsense mediated mRNA decay or to formation of a truncated protein, we regard our variant as a likely pathogenic mutation. Our results are in agreement with previous observation of loss-of-function mutations adding a pathogenic splice variant to the SF3B4 mutational spectrum.

**E-P11.29****Identification of a de novo novel missense mutation in SMARCA2 gene in Turkish girl with a less affected phenotype of Nicolaides-Baraitser Syndrome**

K. Karaer<sup>1</sup>, Z. Yuksel<sup>2</sup>,

<sup>1</sup>Dr.Ersin Arslan Training and Research Hospital Department of Medical Genetic, Gaziantep, Turkey, <sup>2</sup>Medical Genetics Clinic, Women and Children Hospital, Mersin, Turkey.

Nicolaides-Baraitser syndrome (NCBRS) is characterized by sparse scalp hair, characteristic coarse facial features, prominence of the interphalangeal joints and distal phalanges, seizures and speech delay with developmental delay/intellectual disability. Recent studies reported patients with NCBRS

having mutations in SMARCA2 gene and inherited in autosomal dominant manner.

A 3 years-5 months-old girl was referred to genetic counselling because of mild intellectual disability and dysmorphic features. She was borned to healthy non-consanguineous parents. The child had hair loss, coarse facial features (prominent eyes, prominent and thick eyebrows, wide and open mouth) and seizures. The family history is negative. A mutation analysis of SMARCA2 gene was requested. The results revealed a previously unpublished sequence variant a missense c.3389G>T (p.G1130V) in a heterozygous state. In silico analysis (Mutation Taster, SIFT and PolyPhen) predicts this mutation as potentially deleterious. Sequencing of 24th exon of SMARCA2 gene of both parents was performed, but the variant was not found, confirming a de novo event.

Thus, clinical data of this family were presented by moderate or mild form of the disease. Functional studies and more reports are required to understand for the affect of the mutation and genotype-phenotype correlation.

## E-P11.30

### Noonan syndrome and spinal muscular atrophy - an unusual combination

*N. Beznakow<sup>1</sup>, A. Potulska - Chromik<sup>2</sup>, A. Abramowicz<sup>1</sup>, A. Landowska<sup>1</sup>, M. Gos<sup>1</sup>, A. Kostera - Pruszczyk<sup>2</sup>, E. Obersztyn<sup>1</sup>;*

<sup>1</sup>*Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland, Warsaw, Poland, <sup>2</sup>Department of Neurology, Medical University of Warsaw, Warsaw, Poland.*

We report an unusual combination of Noonan syndrome (NS) and spinal muscular atrophy (SMA) diagnosed in a 17-year-old boy admitted to the Department of Neurology because of gait disturbance and leg tremors. He was born by C-section at 36 weeks of gestation to non - consanguineous parents with unremarkable family history. His birth weight was 3090g (50-75pc), body length 55 cm (>95pc) and (OFC) 33 cm (25-50pc). Lymphedema of the dorsal part of the hand and foot were found after birth. Psychomotor development was normal, however, mild intellectual disability and learning difficulties were noted at school age. On physical examination at age of 17 years he presented with short stature (body height 165,4 cm (-2,02 SD), microcephaly (head circumference 52,8 cm (-2,89 SD), pulmonary valve stenosis, webbed neck, widely spaced nipples, pectus excavatum, scoliosis, myopia, nystagmus, easy bruising and delayed puberty. Dysmorphic features (ptosis, bulbous nasal tip, pointed chin, protruding ears, tall forehead) were typical for NS. Neurological examination revealed proximal and distal muscle weakness and atrophy, foot deformity and walking difficulties disturbances. EMG and muscle biopsy demonstrated neurogenic changes. The Sanger sequencing revealed the presence of known pathogenic -c.922A>G (p.Asn308Asp) mutation in PTPN11. Also, the homozygous deletion of SMN1 exon 7 was found with PCR-RFLP technique. Therefore, the clinical diagnosis of both Noonan syndrome and spinal muscular atrophy were confirmed and to our knowledge this is the first report of NS associated with SMA.

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## E-P11.31

### Noonan-like syndrome with loose anagen hair identified by exome sequencing

*M. Miramar<sup>1</sup>, A. Rodríguez<sup>1</sup>, S. Izquierdo<sup>1</sup>, A. Lasierra<sup>1</sup>, M. Alcaine<sup>1</sup>, J. González<sup>1</sup>, J. Peña<sup>1</sup>, J. López<sup>1</sup>, J. Botet<sup>2</sup>, S. Alvarez<sup>2</sup>;*

<sup>1</sup>*Hospital Universitario Miguel Servet, Zaragoza, Spain, <sup>2</sup>Nimgenetics, Madrid, Spain.*

Introduction: Noonan-like syndrome with loose anagen hair (NS/LAH; OMIM 607721) is a RASopathy characterized by craniofacial features resembling Noonan syndrome, cardiac defects (dysplasia of the mitral valve and septal defects), cognitive deficits and behavioral issues, reduced growth, darkly pigmented skin and an unique combination of ectodermal anomalies. A distinctive trait of NS/LAH is its association with easily pluckable, slow growing, sparse, and thin hair. This rare condition is due to the invariant c.4A > G missense (p.Ser2Gly) change in SHOC2 gene, located in 10q25. This gene encodes a protein that consists almost entirely of leucine-rich repeats, a domain implicated in protein-protein interactions. The protein acts in the RAS/ERK MAP kinase signaling cascade. The p.Ser2Gly mutation creates a new recognition site for N-terminal myristylation, causing aberrant targeting of SHOC2 protein to the plasma membrane and impaired translocation to the nucleus upon stimulation with growth factor. Here we report one NS/LAH case identified by exome sequencing.

Materials and methods: The patient was a seven years old female with psychomotor delay and facial dysmorphisms (easily pluckable, sparse and thin yellow hair, relative macrocephaly and low-set ears), failure to thrive (with GH treatment), atrial septal defect, skin anomalies and epiphyseal dyspla-

sia. An exome study was perfomed by an external laboratory (Nimgenetics, Madrid).

Results: The analysis of clinical exome (5678 genes) revealed the pathogenic c.4A>G mutation in SHOC2 gene associated with NS/LAH.

Conclusions: The present study emphasizes the clinical utility of exome sequencing for rare diseases and it broadens the phenotype spectrum associated with NS/LAH syndrome.

## E-P11.33

### 3 year old girl with a pure de novo 46 Mb duplication of 3p21.31

*K. Vinorum, S. Keim, M. Fannemel;*  
*Oslo University Hospital, Oslo, Norway.*

A 3 year old girl with a de novo partial 3p trisomy (karyotype 4, XX, der (14), t(3;14)(p21.31;p11), confirmed by array CGH; arr3pter21.31(248593-46581062)x3. Born to healthy, unrelated Norwegian parents. Normal pregnancy until gestation week 39 when the mother reported reduced fetal movement. Ultrasound revealed hydrocephalus and hydronephrosis. Sectio was performed due to poor CTG.

BW 25th percentile, BL 75th percentile and HC 75th percentile. The patient had ASD, perimembranous VSD and patent ductus arteriosus. She developed heart failure and had heart surgery at two weeks of age. Hydrocephalus was confirmed and she had an increasing head circumference to 97,5th percentile at the age of 2 months. Her weight and length were at the 25th percentile. MRI of the brain revealed bilateral arachnoidal cysts and accumulation of extracerebral fluid in both lateral ventricles which has been shunted. She has hydronephrosis on right hand side and multiple cysts on left kidney. At the age of 3 years, her weight and length has dropped to 2,5th percentile, and the head circumference normalised (50th percentile).

The patient has severe delayed psychomotor development. She can walk with support and can say a couple of words. She is dysmorphic with hypertelorism, bilateral coloboma, wide mouth and exaggerated Cupids bow. The findings are consistent with previous publications of other partial trisomy 3p patients. Pure trisomy 3p is rare and described in only very few cases.

## E-P11.35

### The use of different types of chromosomal analyses in clarification of aetiology of a plurimalformative syndrome

*M. Grămescu<sup>1</sup>, L. Cabăr<sup>1</sup>, M. Păncaru<sup>1</sup>, L. Butnariu<sup>1</sup>, E. Braha<sup>1</sup>, R. Popescu<sup>1</sup>, S. Popa<sup>1</sup>, C. Rusu<sup>1</sup>, V. Plăiăsu<sup>2</sup>, V. Martinicu<sup>3</sup>, L. Păduraru<sup>1</sup>, M. Stamatin<sup>1</sup>, E. Gorduza<sup>1</sup>;*

<sup>1</sup>*“Grigore T. Popa” University of Medicine and Pharmacy Iași, Iași, Romania, <sup>2</sup>National Institutes for Mother and Child Bucharest, Romania, Bucharest, Romania, <sup>3</sup>Cuza Vodă“ Obstetrics and Gynecology Hospital, Iași, Romania.*

We present a boy born at 35 WA with IUGR, after a pregnancy without medical follow-up. Anthropometric data - W - 2100 gr, H - 44 cm, OFC - 29 cm - confirmed growth retardation. The boy presented a plurimalformative syndrome characterised by: microcephaly, dolicocephaly, broad, flattened nasal root, micrognathia, abnormal years, abnormal big toe, micropenis, atrioventricular defect. To clarify the aetiology of plurimalformative syndrome imposed the chromosomal analyse, we made GTG banding chromosomal analyse. The chromosomal formula was 46,X,Yqh+,1qh+,der(13;18) (q12;p11.2). We applied the C banding that confirmed the presence of one centromere. To clarify the chromosomal anomaly, we applied FISH analyse with Metasystem probes for prenatal detection of aneuploidy. Probes for chromosomes 13 and 21 are locus specific, while probe for chromosome 18 is centromeric. The analyse of derivative chromosome confirmed the presence of two fluorescent signals: one blue (specific for chromosome 18) and one green (specific for chromosome 13). Also, we made FISH analyse with Cytocell probes for subtelomeric region of 18p (74G18) and 18q (dj964M9) and we found on the derivative chromosome the absence of 18p probe and the presence of 18q probe in a normal position at one end of derivative chromosome. The analyses made by us confirmed the association of a cvasicomplete trisomy 18 with a small monosomy 13. In conclusion, this case shows the importance of different type of chromosomal analyses for confirmation of aetiology in plurimalformative syndrome. This study was supported by funding of PN-II-PT-PCCA-2013-4-133 Program of UEFISCDI (National Romanian organism of research).

**E-P11.37****The prevalence of chromosome abnormalities in postnatally diagnosed cases of Thai population**

P. Sa-ingrum, P. Niamrot, S. Dokduang, W. Buchachat, R. Parinayok, B.

Rerkamnuaychoke;

Human Genetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

**Introduction:** Chromosome abnormalities include missing, extra, or irregular portion of chromosome material. There are two basic groups: numerical and structural abnormalities. Postnatal cytogenetic testing is routinely used on: (1) patients with suspected aneuploidy, (2) phenotypically normal individuals with repeated pregnancy loss or (3) family history of chromosome rearrangement. The aim of this study is to describe the prevalence of various types of chromosome abnormalities in postnatal cases.

**Materials and Methods:** In retrospective study at Ramathibodi hospital over a period from 2013-2015, chromosome abnormality data were derived from postnatal cases. Blood samples of 1,012 patients requested for cytogenetic analysis were cultured and chromosome preparation was performed. Karyotype analysis was done on 20 G-banded metaphase spreads.

**Results:** The chromosome abnormalities were present in 173 patients (17.09%). Among these, there were 102 female and 71 male. The autosomal chromosome aneuploidies were identified in 45 cases (26.01%). They were common aneuploidies: trisomy 13, 18 and 21. Sex chromosome abnormalities of Turner syndrome and Klinefelter syndrome patients were detected in 66 cases (38.15%). The structural chromosome abnormalities including deletion, duplication, translocation or inversion were found in 62 cases (35.84%).

**Conclusions:** We determined the frequency of chromosomal aberration among different groups of referrals suspected of chromosomal abnormalities. Our data provides cytogenetic information of Thai patients. Our data is important to support clinician decision and plan for patient care management.

**E-P11.38****A case of Noonan syndrome with coarctation of aorta**B. S. Eklioglu<sup>1</sup>, M. Balasar<sup>2</sup>, P. Taşdemir<sup>2</sup>, M. E. Atabek<sup>1</sup>

<sup>1</sup>Necmettin Erbakan University Medical Faculty, Department of Pediatric Endocrinology, KONYA, Turkey, <sup>2</sup>Necmettin Erbakan University Medical Faculty, Department of Medical Genetics, KONYA, Turkey.

**Introduction:** Noonan syndrome (NS) is characterized by short stature, congenital heart defect, developmental delay of variable degree, broad or webbed neck, superior pectus carinatum or inferior pectus excavatum, cryptorchidism and characteristic facies. This disorder has an autosomal dominant inheritance pattern. The incidence of Noonan syndrome is estimated to be 1/1000-2500 live births. Despite being one of the most common seen in rare diseases, Noonan Syndrome can not be diagnosed with sufficient frequency. Our case was referred to the hospital because of growth retardation. Physical examination findings were prominent-low set ears, high arched palate, downslanting palpebral fissures, epicanthal folds, low nasal bridge, upturned nose, bilateral ptosis, strabismus and pectus excavatum. Measurements of height, weight and head circumference were under 3 percentile. Nearly minimal coarctation of the aorta was defined in echocardiography. Neuro-motor and endocrinological examination was normal. **Materials and Methods:** Conventional cytogenetics analysis and PTPN11 gene mutation analysis were performed. **Results:** The result of karyotype analysis was 46,XY. The patient was diagnosed as Noonan Syndrome. PTPN11 gene mutation analysis was performed. Heterozygous mutations in 13.exon were identified [p.Pro491Ser (c.1471C>T)]. His father also had similar clinical findings. PTPN11 gene mutation analysis was planned for his father. Genetic counseling was given to family. **Conclusions:** PTPN11 gene mutation was identified in 50% of affected individuals. PTPN11 gene mutations increase the risk of malignancy a threefold in Noonan Syndrome. This case is presented here in order to emphasize that Noonan syndrome should be considered when the patient is male, short statured and suffering from coarctation of the aorta.

**E-P11.39****Rubinstein-Taybi: an 8-year follow-up of a male patient**N. O. Dávalos Rodríguez<sup>1</sup>, S. A. Alonso-Barragán<sup>1</sup>, M. A. Aceves-Aceves<sup>1</sup>, I. M. Salazar-Dávalos<sup>1</sup>, M. G. González-Mercado<sup>1,2</sup>, J. J. Magallanes-Ordoñez<sup>2</sup>, C. A. Ramírez-Aréchiga<sup>4</sup>, D. García-Cruz<sup>1</sup>, I. P. Dávalos-Rodríguez<sup>1,2</sup>

<sup>1</sup>Instituto de Genética Humana Dr. Enrique Corona, CUCS, Universidad de Guadalajara, Guadalajara, Mexico, <sup>2</sup>División de Genética, CIBO, IMSS, Guadalajara, Mexico,

<sup>3</sup>Doctorado en Farmacología, CUCS, Universidad de Guadalajara, Guadalajara, Mexico,

<sup>4</sup>Departamento de Bienestar y Desarrollo Sustentable, Centro Universitario del Norte, UdeG, Colotlán, Mexico.

**Introduction:** The syndrome Rubinstein-Taybi (RSTS) is an autosomal dominant syndrome. RSTS1 is caused by heterozygous mutation. RSTS is cha-

racterized by mental retardation, delayed growth, microcephaly, facial dysmorphia, broad thumbs and first toes, among others. Approximately 99% of RSTS occur sporadically. The birth prevalence is 1/125000 and diagnosis is based in clinical characteristics in most cases (Milani et al, 2015).

**Objective:** To present an 8- year follow- up of a male patient with RSTS.

**Case report:** We describe a 13 years old male who at birth was diagnosed with congenital heart disease and bilateral undescended testes. At age one he is diagnosed with strabismus which was surgically corrected at 3. At 2 an orchiopexy was performed. At 4 he presented psychomotor development, generalized hypertrichosis, facial dysmorphological features, allergic rhinitis, intellectual disability, expressive language disorder and hetero aggressive behavior. Cranial CT scan, renal and abdominal ultrasound were normal. Physical examination showed low frontal hairline, arched thick eyebrows, prominent baked nose, micrognathia, high-arched palate and dental malocclusion, low set dysplastic ears, asymmetric thorax, broad thumbs and big toes. At 6 cardiological evaluation was normal and at 8 he continues with allergic rhinitis and conduct disorders.

**Conclusions:** Clinical findings permit the diagnosis of RSTS in this patient. Multicenter studies are focused in establishing standard diagnostic criteria, providing professional management and follow-up care of RSTS (Milani et al. 2015). RSTS can present brain malignant tumours and hematological disorders. Early diagnosis of RSTS permits a multidisciplinary approach, optimal follow-up and opportune treatment to prevent complications.

**E-P11.41****Newborn with Smith-Magenis syndrome**

E. Simarro Rueda, G. M. Varo Sánchez, M. L. Quintanilla Mata, A. M. Gómez Pastor, L. Navarro Casado; Hospital General Universitario de Albacete, Albacete, Spain.

**Introduction:** Smith-Magenis syndrome is a complex disorder that causes mental retardation of varying severity along with a number of congenital anomalies. Its prevalence is 1/15,000 newborns.

We present a patient with 17 days old to perform a karyotype in peripheral blood. She presents a peculiar phenotype: midface hypoplasia, epicanthus and wide nasal root, short and broad hands and feet, left single palmar crease.

**Material and methods:** Chromosomal analysis were performed on peripheral blood lymphocytes. Fluorescent in situ hybridization (FISH) studies and microarray CGH method were applied in order to confirm the chromosomal deletion.

**Results:** The karyotype analysis was 46,XX,del(17)(p11.2).ish17p11.2(LSI SMSx1), which corresponds to a woman with Smith-Magenis syndrome. Fluorescent in situ hybridization studies (FISH: LSI SMS Sp Orange/LSI RARA Sp Green) and microarray CGH methods confirm the deletion.

**Conclusions:** Patient with Smith-Magenis syndrome (17p11 deletion) causes global developmental delay. Family chromosomal rearrangements involving a deletion in 17p11.2 are rare. The risk of recurrence in another pregnancy would be low, at around 1 % for germline mosaicism.

Despite the difficulty of the karyotype to detect microdeletions, in this case we could identify the microdeletion by this method, which it was subsequently confirmed by FISH and microarray CGH methods.

**E-P11.44****The Clinical Characterization of a Patient with 4p15 Deletion**S. Zeybek<sup>1</sup>, G. Cetin<sup>1</sup>, V. Caner<sup>1</sup>, M. Ozturk<sup>1</sup>, C. Semerci<sup>1</sup>, G. Bagci<sup>1</sup>, H. Ergin<sup>2</sup>

<sup>1</sup>Pamukkale University, School of Medicine, Department of Medical Genetics, Denizli, Turkey, <sup>2</sup>Pamukkale University, School of Medicine, Department of Pediatrics, Denizli, Turkey.

The Wolf-Hirschhorn syndrome (WHS) is characterized by severe growth and mental retardation, closure defects (cleft lip or palate, coloboma of the eye, and cardiac septal defects) and typical craniofacial features consisting of 'Greek warrior helmet' appearance of the nose, microcephaly, high anterior hairline with prominent glabella, widely spaced eyes, downturned corners of the mouth, and poorly formed ears with pits/tags. The diagnosis of Wolf-Hirschhorn syndrome is established by detection of a heterozygous deletion of the Wolf-Hirschhorn syndrome critical region (WHSCR) within 4p16.3 at ~1.4-1.9 Mb from the terminus. The most common breakpoint is 4p15 in deletions of 4p and therefore the syndrome is described under this aberration. We aimed to compare the Wolf-Hirschhorn contiguous gene syndrome and 4p deletion syndrome features with the patient presented here, who was a one month old girl with hypotonia, congenital heart disease, microcephaly, microphthalmia, coloboma of the right iris, incomplete cleft palate, club foot, prominent perineal raphe and typical craniofacial features of WHS such as Greek warrior helmet appearance; on the basis of breakpoint regions.

## E-P11.45

**Sex reversal in a severely malformed newborn with Xp duplication due to the maternal X;10 translocation**

*E. Sukarova-Angelovska, M. Kocova, G. Ilieva, V. Anastasovska;  
University Pediatric Clinic, Skopje, Macedonia, The Former Yugoslav Republic of*

Chromosomal rearrangements represent a rare cause of sex reversal, involving sex chromosomes and autosomes (deletions of 9p, 10q, 12q, etc), all with variable clinical presentation. Translocation between Xp and autosome disables inactivation of the translocated portion of X chromosome, and over-expresses the DAX1 gene located on DDS region.

A severely malformed newborn was born after a complicated pregnancy. Dysmorphic features were distinct, including microcephaly, wide forehead, narrow eyelids, wide nose, micrognathia, unruly hair, narrow shoulders, arthrogryposis preferably on arms and hypoplastic female external genitalia with no palpable gonads. The baby had dilated aortal root, vesicoureteral reflux and enlarged CNS ventricles. Ultrasonography of the genitalia showed streak mullerian structures and small underdeveloped testes in the lower abdomen. Due to the severe neurological impairment the baby died within the first months. Chromosomal finding represents translocation between chromosomes X and 10, 46, XY, t(X; 10) with a breakpoint on Xp21, which was inherited from the mother. FISH study including Xp and Xq probes confirmed duplication of Xp. Subtelomeric deletion of 10q was not found.

Duplication of dosage sensitive region on Xp is well established cause for male to female sex reversal. A variety of clinical presentations of Xp were described in the literature, mostly depending on the involved autosome. Loss of the autosomal segment of chromosome 10 was not found in our patient, therefore we can assume that overexpression of other genes on Xp on PAR1 region could be the cause for additional features of our patient (radioulnar synostosis, microcephaly, severe dysmorphism).

**E-P12 Cancer genetics**

## E-P12.001

**Recurrent and rare chromosomal abnormalities in acute leukemias - a 5 years study**

*S. Papuc<sup>1</sup>, A. Tutulan-Cunita<sup>1</sup>, M. Cristea<sup>1</sup>, I. Borcan<sup>1</sup>, H. Bumbea<sup>2</sup>, D. Cisleanu<sup>2</sup>, M. Tevet<sup>3</sup>, V. Popov<sup>3</sup>, C. Dragan<sup>3</sup>, N. Berbec<sup>4</sup>, S. Angelescu<sup>4</sup>, A. Vladareanu<sup>4</sup>, A. Arghir<sup>4</sup>;  
<sup>1</sup>Victor Babes National Institute of Pathology, Bucharest, Romania, <sup>2</sup>Emergency University Clinical Hospital, Bucharest, Romania, <sup>3</sup>Colentina Clinical Hospital, Bucharest, Romania, <sup>4</sup>Coltea Clinical Hospital, Bucharest, Romania.*

Acute leukemias (ALs) are a heterogeneous group of disorders characterized by clonal proliferation of lymphoid / myeloid progenitor cells. Many genetic lesions are described across ALs subtypes, from recurrent reciprocal translocation to focal copy number changes and point mutations. The genetic findings, alongside hematologic and immunophenotypic data, provide valuable diagnostic and prognostic information.

We report on the results of genetic investigations in 101 adult patients with acute leukemias referred to our laboratory since 2011.

Seventy seven acute myeloid leukemias (AML), 23 acute lymphoid leukemias (ALL) and one biphenotypic leukemia (BAL) were investigated. Bone marrow aspirate was used for morphological, cytochemical, flow cytometry and chromosomal studies. Cytogenetic investigations were performed on GTG-banded slides. FISH with locus specific probes (commercial and „home-made“) was applied for molecular characterization.

58% of AML patients showed chromosomal anomalies; ~70% of ALL harboured cytogenetic changes, BCR/ABL1 translocation being the most frequently detected (30% of ALL patients). Besides the frequently reported recurrent chromosomal anomalies, with known prognostic impact, some rare or complex abnormalities were detected in our patient group. Among the latter, complex karyotypes with various chromosomes affected, low level amplification of KMT2A through unbalanced translocations and other structural rearrangements (ring chromosome) were identified.

The cytogenetic data contributed to diagnostic refinement (e.g. acute leukemia with recurrent genetic abnormalities), prognosis evaluation, disease monitoring, and guidance of therapeutic strategies (tyrosine-kinase inhibitors in t(9;22) ALL) in our patient group. Our study proves once more the value of genetic testing that informs diagnosis and prognosis in hemato-oncology.

## E-P12.002

**Polymorphism in the lymphotoxin-alpha gene, position +250 (G>A) associated with acute lymphoblastic leukemia in Serbian Children**

*R. Milicevic<sup>1</sup>, L. Brankovic<sup>1</sup>, D. Jugovic<sup>1</sup>, D. Radulovic<sup>1</sup>, H. Stamenovic<sup>1</sup>, A. Milicevic<sup>2</sup>;  
<sup>1</sup>Pediatric Clinic, Nis, Serbia, <sup>2</sup>Institute for clinical pathology and pathologic anatomy, Nis, Serbia.*

Introduction: Lymphotoxin (LT) alpha, a member of the tumor necrosis fac-

tor cytokine superfamily. Tumor necrosis factor alpha (TNF- $\alpha$ ) and Lymphotoxin alpha (LT- $\alpha$ ) have been shown to play an important role in the pathogenesis of lymphoproliferative diseases. Both cytokines regulate cell survival and cell -death in leucemic cells. LT - $\alpha$  has a well defined role in secondary lymphoid organogenesis and it also controls cellular proliferation and differentiation. Since LT alpha is a potent controller of cell functions, it would be of interest to demonstrate that deregulation of LT - $\alpha$  production in acute lymphoblastic leukemia patients is genetically determined. Materials and Methods: We investigated frequencies of polymorphic alleles and a possible association of LT alpha polymorphisms +250(G>A), with increased risk for acute lymphoblastic leukemia (ALL). Genotype was analyzed using the polymerase chain reaction-restriction fragment length polymorphism technique with genomic DNA isolated from peripheral blood lymphocytes. The research was performed on the DNA samples of 75 children suffering from ALL and 46 healthy individuals.

Results: The following genotype frequencies were found: 53% (G/A), 17% (G/G) and 29% (AA) in ALL patient. Genotypes in healthy individuals were: 50% (G/A), 2,2% (G/G), 48% (A/A). The frequencies of AA genotypes were higher in healthy individuals than in children with ALL.

Conclusion: A statistically significant difference was found in the genotype frequencies between ALL patients and controls. Our results point to the association between lymphotoxin- $\alpha$  +250 polymorphism and increased ALL risk in Serbian children.

## E-P12.003

**Modulation of prognosis in acute myeloid leukaemia with concomitant trisomy 4 and double minutes - a molecular mechanism of micronuclei**

*L. L. P. Siu, W. S. Wong, H. F. Wong, K. F. Wong;*

*Department of Pathology, Queen Elizabeth Hospital, Kowloon, Hong Kong.*

Introduction: We described a case of acute myeloid leukaemia (AML) with concomitant trisomy 4, double minutes (dmin) and heterozygous deletion of MYC. The role of the micronuclei in preferentially capturing the dmin and possibly its therapeutic potential in AML with a dmin karyotype was illustrated.

Materials and Methods: A 79-year-old woman presented with leukocytes of  $4.8 \times 10^9/L$  and 9% blasts. Conventional cytogenetics was performed on the bone marrow after overnight synchronised culture. Fluorescence in situ hybridization (FISH) with IGH/MYC/CEP8 tri-colour dual-fusion probe (Vysis) was performed on both cultured cell pellet and May-Grunwald Giemsa stained bone marrow smears.

Results: Karyotypic analysis showed 47,XX,+4,5~80dmin[19]/46,XX[1]. FISH showed MYC amplification in the dmin, but the MYC gene in one of the chromosomes 8 was deleted in all metaphases with presence of dmin. Multiple micronuclei formation by budding around the interphase nuclei was found in the cultured sample. The micronuclei were varied in size, with some being large and harbouring numerous MYC signals. In the myeloblasts of uncultured sample, only occasional micronuclei were found and they were tiny in size.

Conclusion: FISH study has demonstrated the difference in micronuclei formation due to MYC amplification in neat and cultured samples. It is tempting to speculate that the dmin-type micronuclei are formed during the S-phase in synchronized culture as a result of entrapment and elimination of dmin. Further study on cellular differentiation pathway may facilitate the understanding of dmin-type micronuclei in disease modulation.

## E-P12.004

**Acute myeloid leukemia with a rare variant b3a3 (e14a3) BCR-ABL1 fusion transcript**

*D. Kim, S. Shin, S. Lee, J. Choi;*

*Department of Laboratory Medicine, Yonsei University, College of Medicine, Seoul, Korea, Republic of.*

Introduction: BCR-ABL1 fusion transcript is generally known as a recurrent genetic abnormality in chronic myelogenous leukemia and acute lymphoblastic leukemia. Besides, BCR-ABL1 fusion gene has been also reported in acute myeloid leukemia (AML) usually as an additional cytogenetic defect. Here, we report a patient who diagnosed AML with a rare variant b3a3 BCR-ABL1 fusion transcript.

Materials and Methods: A 52-year-old female came to the out-patient clinic for generalized weakness. Peripheral blood analysis showed white blood cell count  $55.97 \times 10^9/L$  with 66% blast; hemoglobin was 8.2 g/dL, platelet count  $105 \times 10^9/L$ . The bone marrow examination showed a hypercellular marrow loaded with large-sized leukemic blasts with moderate amount of granular cytoplasm in 38.3% of all nucleated cells.

**Results:** Molecular genetic analysis using multiplex RT-PCR; Hemavision kit (DNA Technology, Aarhus, Denmark) showed positive in M6B lane with the product of approximately 300 base pairs. To confirm the subtype of *BCR-ABL1* translocation, sequence analysis was performed, and revealed as b3a3 breakpoint. The patient was underwent induction chemotherapy, tyrosine kinase inhibitor therapy and peripheral blood stem cell transplantation; however, relapsed after 2 year and died with systemic infections.

**Conclusions:** *BCR-ABL1* fusion transcript is found in 3% of AML, and known as a poor prognostic factor in AML. To our best knowledge, it is the first case of AML with *BCR-ABL1* b3a3 fusion transcript. In addition, clinical laboratorians should be aware that rare type fusion transcripts such as b3a3 may escape detection when using assays which utilize primers complementary to *ABL1* exon a2.

#### E-P12.005

##### Up-regulation of LATS2 gene associates with acute myeloid leukemia

M. Gholami<sup>1</sup>, S. Manoochehrabadi<sup>1</sup>, H. R. Heidari-Rostami<sup>1</sup>, H. Jalaeekhoo<sup>2</sup>, A. Movafagh<sup>1</sup>, R. Mirfakhraie<sup>2</sup>;

<sup>1</sup>Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of,

<sup>2</sup>Emam Reza Hospital, AJA University of Medical Sciences, Tehran, Iran, Islamic Republic of.

**Introduction:** Acute myeloid leukemia (AML) is the most common acute leukemia in adults, that is heterogeneous with respect to presentation and clinical outcome. Currently, cytogenetic findings represent the most powerful prognostic factor in AML; however, about 35 to 50 percentage of the patients have a normal karyotype. Molecular markers allow precise classification of AML patients. Recent clinical studies have indicated that the expression level of LATS2 correlates with clinical course of some malignancies. The aim of the present study was to analyze the expression level of the LATS2 gene in acute myeloid leukemia (AML) patients. **Materials and methods:** Using quantitative real-time PCR, the expression level of the LATS2 gene was detected in peripheral blood samples from 32 patients with de novo AML and 10 normal controls.

**Results:** LATS2 gene was significantly over-expressed in AML patients compared to normal subjects. Significant LATS2 over-expression was also detected in all FAB types except for the M3.

**Conclusion:** The present work provides the first evidence of the overexpression of LATS2 in AML patients and suggests that the gene might play a role in the disease development and hence may be a potential therapeutic target for AML treatment.

#### E-P12.006

##### Analysis of The Molecular Markers in 49 AML Patients

E. Arslan Ates, O. Kara, A. Türkyılmaz, H. Simşek, M. Söylemez, K. Delil, B. B. Geçkinli, T. Toptaş, P. Ata, A. Arman, A. T. Tuglular, I. Güney;

Marmara University Pendik Training and Research Hospital, Istanbul, Turkey.

**Introduction :** Acute myeloid leukemia is a cancer characterized by overproliferation of immature myeloid cells. Besides chromosomal rearrangements, FLT3, NPM1 and CEBPA mutations are related with prognosis.

**Aim:** The aim of this study is to establish the frequency of CEBPA, NPM1 and FLT3-ITD mutations in our AML group and to evaluate the clinical outcomes of mutation carriers.

**Materials and Methods:** Forty-nine patients who were diagnosed as AML in our University Hospital between 2014-2015 were investigated for FLT3-ITD, NPM1 and CEBPA mutations by Sanger sequencing. Clinical and genetic characteristics of 13 patients having a mutation in at least one of these genes were presented here.

**Results:** We investigated 13 patients aged between 22-73. Bone marrow karyotype analyses of 10 patients were normal whereas Trisomy 8 were detected in two cases and cytogenetic evaluation was not successful in one patient. In molecular analyses FLT3-ITD, NPM1 and CEBPA mutations were detected in five, seven and five patients respectively. Three of these mutations were novel. In the study group, out of four patients who died with a diagnosis of refractory AML, two were carrying FLT3-ITD and NPM1 mutations, one was carrying FLT3-ITD mutation and one was carrying NPM1 mutation. **Conclusions:** In this study 13 of 49 patients had a mutation at least in one of FLT3 (%10.2) , NPM1 (%14.2), CEBPA (%10.2) genes. To further understand the genotype-phenotype correlation in terms of these genes, we present the clinical and genetic evaluations to contribute to larger studies.

#### E-P12.007

##### Two different nucleotide substitutions of APC Gene in a family with familial adenomatous polyposis

B. Eser, M. Yıldız;

Balıkesir University, Balıkesir, Turkey.

**Introduction:** Familial Adenomatous Polyposis (FAP) is an autosomal dominant syndrome leading to colorectal cancer. This disease appears as a result of germline mutation in Adenomatous Polyposis Coli (APC) gene. Although many studies have been conducted, the genotype-phenotype correlation between APC gene mutations and colorectal and extracolonic involvements of the disease could not be clearly detected. The aim of the present study is to report the association between two different nucleotide substitution detected in a family with FAP and phenotypes of the family members.

**Materials and Methods:** Clinical and colonoscopic data of the proband with FAP and family members were collected. DNA sequencing of the APC gene was applied to the family members.

**Results:** In the proband with FAP phenotype and extracolonic involvement, p.His1172Gln (c.3516delT) was detected in exon 15 of the APC gene. Furthermore, p.His1172Gln (c.3516delT) and in addition to this mutation, p.Met1413Val (c.4237 A>G) were detected in exon 15 in both daughters (which have FAP and attenuated FAP) of the proband. Similarly, p.Met1413Val (c.4237 A>G) nucleotide change was found in the mother of the girls. However, colonoscopic findings of the mother were normal.

**Conclusions:** Single nucleotide change in codon 1413 may be a polymorphic variant. However, we believe that deletion T in codon 1172 of APC gene is associated with FAP, attenuated FAP and extracolonic FAP involvement. Along with common use of genetic tests in the clinical practice, genotype-phenotype correlation may be recognized better and useful for early diagnosis and prevention of familial cancer syndromes.

#### E-P12.008

##### Gene-expression patterns in relapsed B-cell acute lymphoblastic leukemia and potential therapeutic targets

Y. Erbilgin<sup>1</sup>, B. Islek<sup>2</sup>, S. Firtina<sup>2</sup>, O. Hatırnaz Ng<sup>1</sup>, A. Inal<sup>3</sup>, P. Dogan<sup>3</sup>, T. Celkan<sup>4</sup>, Z. Karakas<sup>5</sup>, N. Sarper<sup>6</sup>, M. Sayitoglu<sup>1</sup>, U. Ozbek<sup>1</sup>;

<sup>1</sup>Department of Genetics, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey, <sup>2</sup>Department of Genetics, Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey, <sup>3</sup>Department of Genetics and Bioengineering, Istanbul Bilgi University, Istanbul, Turkey, <sup>4</sup>Department of Pediatric Hematology Oncology, Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey, <sup>5</sup>Department of Pediatric Hematology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey, <sup>6</sup>Pediatric Hematology Division, Kocaeli Medical Faculty, Kocaeli University, Istanbul, Turkey.

**Introduction:** Relapsed acute lymphoblastic leukemia (ALL) ranks as the fourth most common childhood malignancy. To identify targets for new therapies, a thorough understanding of the genetic lesions contributing to establishment of the leukemic clone and resistance to therapy is required. The goals of the study were to provide an explanation for the observed differences in outcome among patients who relapse early and to define high-risk patients much better.

**Materials and Methods:** We examined gene-expression profiles in 20 childhood B-cell ALL and healthy B-cell subsets by using "Illumina HumanHT-12 v4 Expression BeadChip" technology. According to the differential analysis, selected eight genes had been validated in a larger B-ALL cohort (n=81 patients) by using Quantitative Real-Time PCR.

**Results and Conclusions:** Diagnose patients who had relapse showed differential expression in cell cycle, apoptosis, purine/pyrimidine metabolism, and different cancer pathways. Many of these pathways have been implicated in tumorigenesis previously and are attractive targets for intervention strategies. After the validation process, aberrant PMAIP1 and RASD1 expression had been linked with relapsed. PMAIP1 is related with ERK Signaling and apoptosis, RASD1 is related with MAPK targets. However, further studies are required to understand the impact of RASD1 and PMAIP1 genes in drug resistance in B-cell ALL.

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**E-P12.009****Amplification of chromosome 21 in childhood all is associated with a poor outcome and is often without a TEL/AML1 fusion: report of 115 patients from northern Turkey**

H. Mutlu Albayrak<sup>1</sup>, G. Ogur<sup>1</sup>, S. Ozdemir<sup>2</sup>, U. Abur<sup>1</sup>, O. Akar<sup>1</sup>, E. Altundag<sup>1</sup>, A. Yilmaz<sup>1</sup>, C. Albayrak<sup>1</sup>;

<sup>1</sup>Ondokuz Mayis University Medical Faculty Department Of Genetics, Samsun, Turkey,

<sup>2</sup>Ondokuz Mayis University Medical Faculty Department Of Pediatrics, Samsun, Turkey.

**Introduction.** Leukemia is the most common form of childhood cancer. Genetic analysis holds a true prognostic value for childhood leukemia. The aim of this study was to evaluate the impact of cytogenetics and fluorescent in situ hybridization (FISH) analysis in childhood ALL.

**Materials-Methods.** 115 childhood ALL patients were evaluated for cytogenetics and FISH ( BCR/ABL, TEL/AML1 and MLL)

**Results.** Cytogenetic aberration was found in 54.4% of patients. Hyperdiploidy rate was %12 with gains for chromosomes 4, 14 and 21. Hypodiploidy rate was 1.7%. FISH aberration was found in 52.2% of patients. Polisomy 21 (amplification 21) was the most frequent anomaly (25.2%). TEL/AML1 fusion rate was 13.9% whereas BCR/ABL fusion rate was 3.5%. TEL/AML1 fusion was found less in group with polisomy 21. MLL rearrangement rate was 5.4%.

No relapse was found in patients with TEL/AML 1 fusion. One patient died of sepsis. Overall survival rate of patients with positive TEL/AML1 fusion was high.

Mortality rate of the patients with a BRC/ABL fusion, was higher (50%); the survival rate was shorter (25%) when compared to those who did not have the fusion. The group with amplification 21 also showed a higher rate of relapse (17%) and mortality (10%) and a shorter survival rate.

**Conclusion.** Polysomy 21 is not rare in childhood ALL; it is a poor prognostic indicator with rates not as high but similar to bcr/abl fusion. TEL/AML1 predicts a good prognosis. It was of interest to note that existence of polysomy 21 decreased the rate of TEL/AML1 fusion.

**E-P12.010****A case of Trisomy 4 with AML M5**

P. Oltulu<sup>1</sup>, P. Taşdemir<sup>2</sup>, M. Balasar<sup>2</sup>, H. Çipil<sup>3</sup>;

<sup>1</sup>Necmettin Erbakan University Medical Faculty, Department of Pathology, KONYA, Turkey, <sup>2</sup>Necmettin Erbakan University Medical Faculty, Department of Medical Genetics, KONYA, Turkey, <sup>3</sup>Necmettin Erbakan University Medical Faculty, Department of Hematology, KONYA, Turkey.

**Introduction:** Acute myeloid leukemia (AML) characterized by clonal proliferation of myeloid precursor cells is a type of hematopoietic neoplasm. AML constitutes 80% of adult acute leukemia. The average age for diagnosis in adults is 65 and its incidence increases with advanced ages. Solely trisomy 4 abnormality is a rare cytogenetic finding in AML cases and until today it has been reported in cases with AML M1, M2 and M4. We presented here a case of AML M5 determined solely trisomy 4 in cytogenetic analysis.

**Materials and Methods:** The patient's bone marrow tissue was performed conventional cytogenetic analysis and AML-MDS FISH Panel (del5q, PML/RAR $\alpha$ , del P53, AML1/ETO, MLL, 7q del, CBF $\beta$ /MYH11, 20q del) (Cytocell, UK), 4p16.3 and 4qter FISH probes.

**Results:** The female patient has admitted to the hospital because of extreme weakness and pain. She was 72 years old and her peripheral blood count analysis were abnormal. She was diagnosed as Acute Monocytic Monoblastic Leukemia(AML M5) with histopathological examination of bone marrow. AML-MDS FISH Panel was normal. The result of bone marrow cytogenetic analysis was 47,XX,+4[5] /46,XX[13]. 4p16.3 and 4qter FISH probes were performed, thus trisomy 4 was confirmed. The patient was taken to the intensive care unit due to worsening in her general medical condition and died within 2 days.

**Conclusions:** Some cytogenetic abnormalities is associated with prognosis in AML. The prognostic significance of solely trisomy 4 abnormalities in AML patients are unknown and as far as we know trisomy 4 abnormality with AML M5 is presented the first time in the literature.

**E-P12.011****Molecular cytogenetics findings of clinically suspected acute myeloid leukemia and/or myelodysplastic syndrome (AML/MDS) patients**

B. Sarıkepe<sup>1</sup>, S. Zeybek<sup>1</sup>, G. Gonen<sup>1</sup>, Y. Eyüboğlu Tanrıverdi<sup>1</sup>, O. Boz<sup>1</sup>, E. Ceylan<sup>1</sup>, E. Tepeli<sup>1</sup>, G. Çetin<sup>1</sup>, I. Sarı<sup>2</sup>, F. Duzcan<sup>1</sup>, V. Caner<sup>1</sup>;

<sup>1</sup>Pamukkale University School of Medicine Department of Medical Genetics, Denizli, Turkey, <sup>2</sup>Pamukkale University School of Medicine Department of Hematology, Denizli, Turkey.

Acute myeloid leukemia (AML) is a heterogeneous group of aggressive neoplasms and the most common type of adult acute leukemia, with a median

age of 67 years at diagnosis. Since high quality metaphase spreads are hard to obtain from bone marrow samples of the patients with AML/MDS it is quite difficult to detect the chromosomal abnormalities in AML/MDS using conventional cytogenetic analysis with standard G-banding. Fluorescence in situ hybridization (FISH) is a very simple and useful technique that is applied by many cytogenetics laboratories around the world for routine diagnosis of AML/MDS.

Here we report the FISH results of 185 patients who were referred to our laboratory with pre-diagnosis of AML/MDS between January 2014 and January 2016. A total of 141 patients had normal FISH results while the most frequent abnormalities were MLL translocation (10/185, 5.4%), PML/RARA translocation (4/185, 2.1%), and trisomy 8 (4/185, 2.1%). We also detected complex rearrangements in five patients (2.7%). Moreover, we analyzed the relation between chromosomal abnormalities and clinicopathological parameters.

In conclusion, a significant proportion of AML/MDS patients with normal karyotype has clones of cytogenetically abnormal cells which may play an important role in the development and/or progression of the disease. It is important to determine the presence or absence of specific chromosome and/or gene alterations by FISH analysis for the prognosis and survival of the patients.

**E-P12.012****Aurora kinase inhibition affects cell cycle and apoptosis in breast cancer**

C. Kayabaşı, B. Özmen Yelken, T. Balci, A. Aşık, S. Gonca, F. Söğütlü, R. Gasimli, S. Yılmaz Süsler, Ç. Biray Avcı, C. Gündüz;

*Ege University Faculty of Medicine, Department of Medical Biology, Izmir, Turkey.*

Breast cancer remains the most common malignancy in women. Dysregulated FGFR is a significant risk factor in breast cancer. Aurora kinases are serine/threonine kinases that play crucial roles in cell division including mitosis checkpoint and chromosomal segregation. CCT137690 is a novel Aurora kinase inhibitor. In this study we aimed to evaluate the anti-cancer effects of CCT137690 in human breast cancer cells.

Cytotoxicity of aurora kinase inhibitor CCT137690 (24 nM-50  $\mu$ M) on estrogen receptor-positive human breast cancer cell line MCF-7 and normal human fibroblasts WI-38 were evaluated with xCELLigence real time cell analysis system. Effects on apoptosis and cell cycle in MCF-7 and WI-38 cells with exposure to IC50 dose of CCT137690 are detected with Annexin V-EGFP Apoptosis Detection Kit and Cycletest Plus DNA Reagent Kit with FACS, respectively.

For MCF-7 and WI-38 cell lines IC50 doses of CCT137690 were found as 4.5  $\mu$ M and 9.19  $\mu$ M, respectively. It was shown that CCT137690 induced apoptosis 3.6 fold in MCF-7 and 2.1 fold in WI-38 compared to untreated control cells. In addition to apoptotic effects, CCT137690 caused cell cycle arrest at G2/M in MCF-7 cells significantly.

In conclusion, inhibition of aurora kinases is a novel approach for treatment of malignant disorders. Considering infinite division capacity of the cancer cells, inhibiting cancer cell cycle in G2/M and leading cancer cells to apoptosis more than normal cells indicates that CCT137690 can be a potential anti-cancer agent for targeting breast cancer cells.

**E-P12.013****Determination of apoptosis and changes on cell cycle genes expressions in HL60 cell line induced with bendamustine**

S. Kırçak<sup>1</sup>, B. Özel<sup>1</sup>, C. Aktan<sup>1</sup>, B. Tezcanlı Kaymaz<sup>1</sup>, Ç. Biray Avcı<sup>1</sup>, B. Kosova<sup>1</sup>, G. Saydam<sup>2</sup>, N. Selvi Günel<sup>1</sup>;

<sup>1</sup>Ege University, Department of Medical Biology, Izmir, Turkey, <sup>2</sup>Ege University, Department of Hematology, Izmir, Turkey.

**Introduction:** Acute promyelocytic leukemia (APL), is a subtype of Acute myeloid leukemia (AML) and characterized by aberrant morphology of promyelocytes. The most specific chromosomal disorder that occurs in APL patients is (15;17) translocation. This translocation produce a PML-RAR $\alpha$  fusion protein. Bendamustine is a water soluble, microcrystalline powder with amphoteric properties and it acts as an alkylating agent and also has purine analog activity. Bendamustine has more moderate toxicity profile than the other alkylating agents. In our study, it is aimed to investigate cytotoxic and apoptotic effects of bendamustine on APL cell line HL60. Also we aimed to evaluate the effect of bendamustine on HL60 cells according to the expression of genes including cell cycle, mitotic catastrophe, apoptosis. **Materials and Methods:** Cytotoxic effect of bendamustine on HL60 cell line was assessed by WST-1 assay, Annexin V and Apodirect Tunnel assay are used for apoptotic effect. Also the effect of bendamustine on expression levels of genes in HL60 was determined by quantitative RT-PCR relatively.

**Results:** Bendamustine significantly induces apoptosis in dose dependent manner on HL60 cells. After the 48 hours treatment with bendamustine, expression levels of apoptosis related CASP7, CASP8, CASP9 genes were upregulated 2.4, 5.7, 6.8 fold in HL60 cells, relatively. Also expressions of mitotic catastrophe related genes Plk1downregulated 2.5 fold and Plk2 which as known as tumor suppressor gene, upregulated 17 fold.

**Conclusion:** Our data suggest that Bendamustine is effective on cell death mechanisms such as apoptosis ve mitotic catastrophe and can be used in supplement APL treatment.

#### E-P12.014

##### Copy number variations associated with tumor progression in invasive bladder cancer

*O. S. Antonova<sup>1</sup>, S. Hadjidekova<sup>1</sup>, Z. Hammoudeh<sup>1</sup>, R. Staneva<sup>1</sup>, V. Hadzhiyska<sup>2</sup>, G. Stamenov<sup>3</sup>, C. Slavov<sup>4</sup>, D. Toncheva<sup>1</sup>*

<sup>1</sup>Department of Medical Genetics, Medical University- Sofia, Sofia, Bulgaria, <sup>2</sup>Clinic of nuclear medicine, University Hospital Aleksandrovska, Sofia, Bulgaria, <sup>3</sup>MBAL Nadezhda, Sofia, Bulgaria, <sup>4</sup>Department of urology, University Hospital Aleksandrovska, Sofia, Bulgaria.

**Background:** The aim of this study was to detect copy number variations (CNVs) and to evaluate their association with tumor progression in non-invasive and muscle-invasive bladder carcinomas in Bulgarian patients.

##### Materials & Methods:

Bladder tumor samples were collected, frozen at -20 °C and histologically confirmed as primary transitional cell carcinomas. The DNA was isolated by phenol-chlorophorm extraction and aCGH analysis was performed on twelve tumors in stages pTa, pT1, pT2, pT2a and pT2b on CytoChip Oligo aCGH, 4x44K format (Illumina). Data was analyzed by BluefuseMulti software v4.2. The clinical significance was further evaluated by literature and database search.

**Results and discussion:** The DNA microarray showed an increased genomic instability and revealed numerous nonspecific chromosomal aberrations in the transitional cell carcinomas - 75 pathogenic deletions and duplications, 3 benign CNVs and 14 variants of uncertain clinical significance. A total of 24 recurring chromosomal regions with pathogenic CNVs were identified. Genomic imbalance in four of these regions, containing more than 200 genes, is present in 50% of the tumors with aberrations (pT1, pT2 and pT2b). A detailed survey was performed for all of these genes and 31 of them were found to be probably associated with the process of tumorigenesis. Of these, 10 are already reported by other scientific groups as candidate-oncogenes for uroepithelial tumours. Our group reports for the first time that genes FLJ46066, LPP, CLDN16, STK3 and TTK could be potential candidate-oncogenes in the progression of bladder cancer.

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#### E-P12.018

##### BRCA1 and BRCA2 mutations in ovarian cancer patients with brain metastases

*T. Dubrovina, A. Semyanikhina, O. Arkhipova, M. Filippova, A. Danishevich, D. Naskhletashvili, L. Lyubchenko, FSBI „Russian cancer research center named after N.N. Blokhin”, Moscow, Russian Federation.*

**Introduction:** Brain metastases from ovarian cancer are a rare event. It occurs in 1-12% cases. Molecular pathways of ovarian cancer metastatic behavior are the subject of scientific and clinical research and still remain unclear. Several studies have shown that the loss of *BRCA1* function may be involved in the phenomenon of brain metastases from ovarian cancer.

**Materials and methods:** To research the implication and frequency of germline mutations in *BRCA1* and *BRCA2* genes we examined their structure in order to detect 185delAG, 4153delA, 5382insC, 3875del4, 3819del5, C61G, 2080delA mutations in *BRCA1* and 6174delT mutation in *BRCA2*. Genotyping was performed on DNA samples extracted from peripheral blood specimens of 11 patients with ovarian cancer and brain metastases using «BRCA SNP genotyping Kit» by DNA-Technology. The median age at diagnosis was 53,2 years. One patient had endometrioid carcinoma, the other had serous adenocarcinoma. 50 % of patients had a family history with malignant tumours.

**Results:** *BRCA1* and *BRCA2* mutations were identified in six cases. The most frequent *BRCA1* 5382insC mutation was detected in four of all cases. One patient had *BRCA1* 4153delA, the other - *BRCA2* 6174delT.

**Conclusion:** Our results confirm the hypothesis that mutations in *BRCA1* and *BRCA2* genes can be implicated in a brain metastasis process. It has been reported that patients with *BRCA*-associated ovarian cancer have a better prognosis and a good therapeutic response in comparison to sporadic cases. There is a possibility that these distinctions correlate with brain metastases at the terminal stage of the disease.

#### E-P12.019

##### Haplotype analysis of common variants in the BRCA1 and BRCA2 genes in Turkey

*B. Sağlam Ada<sup>1</sup>, K. Bilecen<sup>2,3</sup>, K. Yararbaş<sup>4,5</sup>, T. Hatipoğlu<sup>6</sup>, M. Say<sup>7</sup>, C. Tunçer<sup>4</sup>, E. Kavak<sup>8</sup>, A. Tükün<sup>9,7</sup>*

<sup>1</sup>Düzen Laboratories Group Genetic Diagnosis Center, Ankara, Turkey, <sup>2</sup>Düzen Laboratories Group R&D Department, Ankara, Turkey, <sup>3</sup>Konya Food and Agriculture University Department Of Molecular Biology and Genetic Engineering, Konya, Turkey, <sup>4</sup>Düzen Laboratories Group Genetic Diagnosis Center, İstanbul, Turkey, <sup>5</sup>Maltepe University, İstanbul, Turkey, <sup>6</sup>Ankara University Biotechnology Institute, Ankara, Turkey, <sup>7</sup>Düzen Laboratories Group Genetic Diagnosis Center, Ankara, Turkey, <sup>8</sup>Genomize Inc., İstanbul, Turkey, <sup>9</sup>Ankara University School of Medicine Medical Genetics Department, Ankara, Turkey.

Breast cancer is one of the most common cancer types seen in women. Around 10% of overall breast cancer cases have family history. Carriers of BRCA1/BRCA2 germline mutations from families with high cancer risk have been estimated to have a 65-85% lifetime risk of developing breast/ovarian cancer. In addition to pathogenic alterations of BRCA1/BRCA2 genes, several polymorphisms have also been reported. Many of these polymorphisms are shown to have protective or possible causal effect on breast cancer formation. Interestingly, several studies showed that some of these polymorphisms tend to occur together although their effects are opposite. In this study, BRCA1/2 single nucleotide polymorphisms (SNPs) were examined for the identification of haplotypes and to evaluate the prevalence of these haplotypes among sporadic early-onset breast cancer patients, familial breast cancer patients, and healthy high risk females in multiple-ethnic regions of Turkey.

Peripheral blood DNA samples from 271 subjects were investigated by next-generation sequencing (NGS) using Illumina-MiSeq. Genomize-Seq and SeqPilot softwares were used for analysis of NGS data. Mutations in the coding gene sequence were screened and all observed mutations have been checked for their pathogenicity at HGMD. Statistical evaluation was carried out using by using in-house developed Python scripts.

c.4837A>G, c.3113A>G, c.2311T>C, c.4308T>C, c.4485-63C>G, c.-19-115T>C, c.2082C>T, c.3548A>G in BRCA1, and c.426-89T>C, c.1365A>G, c.2971A>G, c.7435+53C>T, c.865A>C, c.425+67A>C in BRCA2 were determined as a haplotype in population of Turkey. Our study indicates a high incidence of one haplotype in BRCA1 gene and one haplotype in BRCA2 gene in our study population.

#### E-P12.020

##### Common BRCA1/2 genes mutations test - rapid but insufficient analysis for HBOC diagnosis

*R. Vainoriene<sup>1</sup>, O. Liaugaudiene<sup>1,2</sup>, V. Asmoniene<sup>1</sup>*

<sup>1</sup>Hospital of Lithuanian University of Health Sciences Kauno klinikos, Kaunas, Lithuania, <sup>2</sup>National Cancer Institute, Vilnius, Lithuania.

**Introduction:** BRCA1/2 genes mutations are the most common cause of hereditary breast and ovarian cancer (HBOC). We present an analysis of mono-institutional cohort of unrelated BRCA1/2 mutations carriers.

**Methods:** We analyzed 230 BRCA1/2 tests for 5 BRCA1 and 1 BRCA2 mutations performed in 2015. Data on 23 BRCA1 mutation carriers were collected. The results were compared with data on Lithuanian and European BRCA1/2 mutation carriers.

**Results:** Three BRCA1 mutations were identified in 10% of patients tested for HBOC (39.1% 4153delA, 34.8% 5382insC, 26.1% 300T>G). Other two BRCA1 mutations (185delAG, 2080delA) and BRCA2 mutation 6174delT were not detected. Among BRCA1 mutation carriers 82.6% have positive family history; 47.8% have breast cancer; 34.8% have triple negative breast cancer; 34.8% have ovarian cancer. A study of Janavicius and al. (2014) identified BRCA1/2 mutations in 29% of patients selected for genetic testing (BRCA1 - 25.9%). The most common BRCA1/2 mutation was also 4153delA - a Baltic founder mutation. Our tested mutations amount for 76.7% of found BRCA1/2 mutation in Lithuanian carriers. European studies of full or nearly full sequence analysis of BRCA1/2 genes revealed higher prevalence of pathogenic mutations in high-risk women (see table 1).

**Conclusion:** Identified mutation rate significantly differs from other Lithuanian and Europe studies and is insufficient. Despite of rapidity and convenience of common mutation testing, full gene sequence analysis is recommended to diagnose HBOC syndrome.

Study	Number of individuals	Prevalence of BRCA1/2 mutations	Type of BRCA1/2 genes analysis	Nationality
Current study	230	10%	Common mutations	Lithuanian
Janavicius and al. 2014	753	29%	Full genes sequences	Lithuanian
Frank et al. 2002	4379	16%	Full genes sequences	European
Krajc et al. 2008	145	39%	Full open reading frame	Slovenian

Konstantopoulou et al. 2008	127	16.5%	Full genes sequences	Greek
Machackova et al. 2008	1010	29.1%	Complete coding sequences and splice sites	Czech
Nedelcu et al. 2002	116	33.6%	Full genes sequences	Italian
Diez et al. 2003	624	26.3%	Full genes sequences	Spanish
Claes et al. 2004	349	21.5%	Complete coding region	Belgian

**E-P12.022****Familiar breast cancer with no phenotype/genotype correlation: case study**

**M. Janikova<sup>1,2</sup>, R. Kratochvilova<sup>1</sup>, H. Synkova<sup>1</sup>, R. Vrtel<sup>1</sup>, R. Vodicka<sup>1</sup>, R. Slavkovsky<sup>3</sup>, T. Veberova<sup>1</sup>, V. Curtisova<sup>1</sup>, M. Prochazka<sup>1</sup>;**

<sup>1</sup>Department of Medical Genetics, University Hospital and Palacky University Olomouc, Olomouc, Czech Republic, <sup>2</sup>Department of Clinical and Molecular Pathology, University Hospital and Palacky University Olomouc, Olomouc, Czech Republic, <sup>3</sup>Institute of Molecular and Translational Medicine, University Hospital and Palacky University Olomouc, Olomouc, Czech Republic.

Introduction: Application of BRCA1/2 testing to clinical praxis provided much information about one of the most frequent familiar cancer - breast and ovarian cancer. Usually, family members carrying pathogenic variant in these genes have higher risk of cancer than non-carriers. These family members are screened in earlier age and more frequently than the rest of population. It can help to distinguish cancer in earlier stages and these patients have bigger chance for successful treatment. Sometimes, phenotype of family members does not fit to their genotype.

Materials and Methods: We searched pathogenic variants in BRCA1/2 genes using massive parallel sequencing. We used BRCA MASTR™ Dx kit (Multiplicom) and platform MiSeq (Illumina). We used Sanger sequencing for confirmation and for testing family members.

Results: We identified previously described pathogenic variant in BRCA1 gene (rs80357609) in mother born in 1950. She developed breast cancer in 1995. Therefore, we tested her daughters for the presence of this variant. We have found that one daughter (born in 1976) carries this variant but she is still without symptoms. The second daughter (born in 1971) does not carry this variant but she developed breast cancer in the same age as her mother.

Conclusions: We identified family with breast cancer history whose family members' genotype does not fully correlate with their phenotype. It might be caused by phenomenon known as phenocopy, which is non-hereditary, environmentally induced trait. It could explain the same phenotype of daughter non-carrying pathogenic variant as her mother with BRCA1 mutation has.

**E-P12.023****Pathogenic splice mutation (c.632-3C>G) in the BRCA2 gene in a family with breast cancer**

**C. D. Castelão<sup>1</sup>, I. R. Silva<sup>1</sup>, M. Carmo-Fonseca<sup>1</sup>, A. Berta<sup>2</sup>;**

<sup>1</sup>Genomed, Lisbon, Portugal, <sup>2</sup>Hospital de Santa Maria, Lisbon, Portugal.

Introduction: Women with inherited inactivating mutations in the tumor suppressor genes BRCA1 or BRCA2 have an increased risk of developing breast and ovarian cancers, while BRCA2 mutation carriers have in addition increased risk for other cancer types including male breast cancer. However, pathogenic mutations in BRCA1 or BRCA2 genes are only detected in approximately 25% of patients with a strong family history, while almost 1,800 distinct sequence variants have been described with uncertain clinical significance.

Methods: The entire coding region and splice site junctions of the BRCA1 and BRCA2 genes were amplified by PCR and analyzed through direct Sanger DNA sequencing.

Results: A female proband diagnosed with breast cancer was selected for BRCA1 and BRCA2 genetic testing according to established criteria. A heterozygous intronic variant within intron 7 of the BRCA2 gene, c.632-3C>G (rs568027879) was identified. The same variant was detected in two other affected family members, one female and one male. In silico analysis performed with multiple tools predicted an impact in the splicing pattern. This variant was not reported in the Breast Cancer Information Core Database (BIC). However, it is described in the Universal Mutation and ClinVar databases as a variant of unknown significance.

Conclusions: We report, for the first time, co-segregation data for a BRCA2 splicing variant in three family members, including one male, diagnosed with primary breast cancer. This strongly suggests that the variant c.632-3C>G is a novel pathogenic splicing mutation.

**E-P12.027****The polymorphisms of genes involved in DNA methylation process among leukemia and breast cancer patients from Ukraine**

**I. Dmytryuk<sup>1</sup>, H. Makukh<sup>1</sup>, M. Thyrkus<sup>1</sup>, N. Kitsera<sup>1</sup>, I. Tsybalyuk<sup>2</sup>;**

<sup>1</sup>Institute of Hereditary Pathology National Academy of Medical Sciences of Ukraine, Lviv, Ukraine, <sup>2</sup>West Ukrainian Specialized Children's Medical Center, Lviv, Ukraine.

Introduction. Aberrant DNA methylation and synthesis are key epigenetic factors in tumorogenesis. Folate metabolizing enzymes may influence the bioavailability of methyl groups and synthesis of nucleotides, whereas DNA methyltransferases are involved in epigenetic regulation of gene expression. We made a suggestion that genetic variants of folate metabolizing enzymes and DNA methyltransferase may lead to differences in susceptibility to cancer development.

Materials and Method. Genotyping of MTHFR 677C>T, MTR 2756A>G, TS 3R2R, TS 3R G>C and DNMT3B -149C>T and DNMT3B -579G>T was performed in 60 patients with leukemia, 90 patients with breast cancer and in 100 healthy persons without cancer pathology in anamnesis. The molecular- genetic analysis was performed by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis. Statistical analysis was conducted by Chi-square tests and odds ratio (OR) calculation.

Results. We did not observe any significant differences in genotype frequencies of the MTHFR, TS and DNMT3B polymorphisms between the cases and controls. The MTR 2756AA genotype frequency was significant higher in patients with breast cancer vs control (0.67 vs 0.50, p= 0.02). The increased risk of breast cancer development was associated with MTR 2756AA genotype (OR=2.00, CI - 95%:1.11 - 3.60) and MTR 2756A allele (OR=1.75, CI - 95%:1.08 - 2.84). The frequency of genotypes and alleles of MTR 2756 A>G polymorphism did not exhibit statistical differences between patient with leukemia compared to control.

Conclusions. Our findings show that West Ukrainian inhabitants carrying at least one MTR 2756A allele have a significantly increased risk of breast cancer.

**E-P12.028****Use of „Myriad My Hereditary Cancer Risk“ test in patients with familial early-onset breast cancer and / or multiple tumors.**

**I. Antonucci<sup>1</sup>, M. Provenzano<sup>1</sup>, L. Sorino<sup>1</sup>, G. Calabrese<sup>2</sup>, G. Palka<sup>2</sup>, L. Stuppi<sup>1</sup>;**

<sup>1</sup>Department of Psychological, Health and Territorial Sciences (DISPUTer), School of Medicine and Health Sciences, "G. d'Annunzio" University, Chieti-Pescara, chieti scalo, Italy, <sup>2</sup>Department of Medical, Oral and Biotechnological Sciences, School of Medicine and Health Sciences, "G. d'Annunzio" University, Chieti-Pescara, chieti scalo, Italy.

Introduction: Breast (BC) and ovarian (OC) cancer are a leading cause of death worldwide and BRCA1/2 genes are the most commonly mutated genes, but additional genes associated with inherited forms are emerging. In the present study we have validated Myriad myRisk Hereditary Cancer panel in a cohort of 18 patients with a family history of BC, using Next Generation Sequencing (NGS).

Materials and Methods: For the screening „Myriad My Hereditary Cancer Risk“, 17 patients were selected on the basis of the following criteria: a) early onset (<40 years); b) evidence of dominant Mendelian transmission : c) bilateral BC o multiple tumors.

Results : The results showed two pathogenetic mutations in 2 patients ( 11,8%) in the BRCA2 and in the APC gene. Furthermore, six patients (35,3%) showed variants of uncertain significance in the following genes: PALB2, PMS2, ATM, BRIP1 e CHEK2.

Conclusions : The preliminary data show that „Myriad My Hereditary Cancer Risk“, panel can be extremely useful and NGS approach is particularly suitable to study patients with BC early and / or family history of multiple cancers.

**E-P12.029****Biological activity of fatty acids in breast and prostate cancer cell lines**

**S. S. Villa Vasquez<sup>1,2</sup>, M. E. Marquez<sup>1,2</sup>, M. Camargo<sup>2</sup>;**

<sup>1</sup>Animal Biotechnology group, Universidad Nacional de Colombia, Medellin, Colombia,

<sup>2</sup>Genetics, Regeneration and Cancer group, Universidad de Antioquia, Medellin, Colombia.

Breast and prostate cancer are the main causes of death worldwide and new therapies or dietary factors influencing cell survival of these cancers are worth investigation. Studies in tumour cell lines suggest that omega-3 fatty acids reduce cell viability, whilst omega-6 increases proliferation. Few studies investigated the genotoxic effect of some acids but a genoprotective effect of eicosapentaenoic acid (EPA) has been found. In this study, we investigated plating efficiency, cytotoxic and genotoxic effect of alpha-linolenic

acid (ALA), EPA, docosahexaenoic acid (DHA), linoleic acid (LA), arachidonic acid (AA) and oleic acid (OA) in prostate and breast cancer cell lines. Cytotoxicity at 25 $\mu$ M, 50 $\mu$ M, 100 $\mu$ M and 150 $\mu$ M of each fatty acid was measured by MTT and Trypan-Blue in MCF-7 and PC-3 cells. Genotoxicity was evaluated by comet assay and plating efficiency done to evaluate capacity colony formation from single cells. Experiments were done in triplicate. DHA, EPA and AA reduce viability in more than 50% of cells. 100% of cytotoxicity was found in PC-3 and MCF-7 cells treated with highest concentrations of EPA and DHA respectively. Unexpectedly, LA had similar effect reducing viability to less than 15%. Relative plating efficiencies lower than 30% were obtained in cells treated with EPA, DHA and AA after 10 days post re-plating. In contrast, EPA caused significant DNA damage ( $p<0,05$ ) in both cell lines and LA in MCF-7 cells. These results suggest that EPA, DHA and AA inhibit proliferation and have long-term effect in tumour cells, therefore their intake can be an adjunctive cancer therapy.

#### E-P12.030

##### Coexistence of t (8; 14 ) and t ( 11; 14 ) translocations in a Burkitt's Lymphoma Patient

S. Karauzum Berker<sup>1</sup>, S. Yakut<sup>1</sup>, K. Yalcin<sup>2</sup>, A. Kupesiz<sup>3</sup>, E. Guler<sup>3</sup>;

<sup>1</sup>Akdeniz University, Faculty of Medicine Department of Medical Biology and Genetics, Antalya, Turkey, <sup>2</sup>Akdeniz University, Faculty of Medicine Department of Pediatric Haematology and Oncology, Antalya, Turkey, <sup>3</sup>Akdeniz University, Faculty of Medicine Department of Haematology and Oncology, Antalya, Turkey.

Non-Hodgkin ,s lymphoma is a subset of the Burkitt's lymphoma and the most common chromosomal abnormality is t (8 ; 14) translocation in this B-cell lymphoma.14-year-old Afghan boy was admitted with increasing swelling in the back of the neck for two months. Burkitt's lymphoma has been reported in a biopsy. Chromosomal analysis was done from the patient's bone marrow aspiration material. His karyotype was designed as 46, XY, t (8 ; 14) ( q24 ; q32 ) [18] / 46, XY, dup (1) ( q23q32 ), t (8 ; 14) ( q24 ; q32 ) [12]. Also t ( 11; 14 ) ( q13 ; q32 ) translocation, often seen in adult mantle cell lymphoma, was found 100 % interphase cells by molecular cytogenetic methods in the same patient. In patients with suspected bone marrow blasts at a rate of 60% blasts and CSF analysis it was considered Stage IV Burkitt's lymphoma and protocol of NHL BFM 95 risk group 4 chemotherapy was started. It has been observed in visible shrinkage of the mass in the neck after a course of treatment. It has been presented for the first time a double hit lymphoma derived from the coexistence of t (8; 14 ) and t ( 11; 14 ) translocations in a child patient with Burkitt's lymphoma diagnosed. Because of the good response to the treatment, clinical and genetic findings of the Burkitt's lymphoma patient will be discussed with the literature.

#### E-P12.031

##### Is Ganoderma lucidum effective for cancer treatment?

H. Ecevit, B. Gogebakan, K. Gunduz, D. Sonmez, M. Izmirli;

Mustafa Kemal University, Hatay, Turkey.

Ganoderma lucidum is a species of mushroom that has been used for a healthier lifestyle, to extend the human life and the treatment of various diseases involving cancer, autoimmune diseases. Lung cancer is the most common cancer among men and women. There are several mechanisms including cell cycle programming and apoptosis cause lung cancer. p21 plays an important role as tumor suppressor gene in cell cycle and Bcl-2, caspase3, caspase8, caspase9 in apoptosis. Therefore, we aimed to evaluate above mentioned parameters. For this purpose, Ganoderma lucidum extract dissolved in DMSO and was prepared in appropriate concentrations in the cell culture medium. A549 was exposed to 0-1000  $\mu$ mol/ml concentrations of the Ganoderma lucidum extract at 24, 48 and 72 hours. Cell viability was evaluated through MTT. The expression levels of genes were evaluated with RT-qPCR. MTT assay results were statistically significant in cells treated with 400  $\mu$ mol/ml concentration of Ganoderma lucidum extract in 24 and 48 hours exposure period than upper concentrations ( $p>0,05$ ; 95% CI:-0,34 to 0,01 and  $p=0,0001$ ; 95% CI:-0,54 to -0,15 for 24 and 48 hours respectively). The expression level of p21 in mentioned treat dose and periods were statistically significant when compared to control group ( $p=0,03$ ; 95% CI:0,004 to 0,06). Consequently, we demonstrated that the Ganoderma lucidum extract was increased A549 cell proliferation and inhibited the release of p21. So, Ganoderma lucidum is shown to have a stimulant effect on cell proliferation via suppressing the p21 expression but not apoptosis.

#### E-P12.032

##### Lactobacilli differentially modulate Wnt/ $\beta$ -catenin, mTOR and HIF-1 pathways in HeLa cell lines

a. abedin do;

Shahid Beheshti University of Medical Sciences, tehran, Iran, Islamic Republic of.

Introduction: *Lactobacilli* are a group of beneficial bacteria whose anti-cancer effects have been evaluated in different cancer cell lines as well as animal models and human subjects. Such anticancer effects can be exerted via different mechanisms such as modulation of immune response as well as inhibition of pathogens colonization. In addition, *lactobacilli* have direct cytotoxic effects against cancer cells which may be exerted through modulation of expression cancer related pathways.

Materials and Methods: In order to find the mechanism of anticancer effects of two *lactobacilli* strains, we analyzed expression of some Wnt/  $\beta$ -catenin, mTOR and HIF-1 pathways genes with real-time PCR in HeLa cell lines following treatment with *Lactobacillus. crispatus* (LC) and *Lactobacillus. rhamnosus* (LR) culture supernatants.

Results: the expression of *CCND1* as a marker of cell proliferation, survival, and angiogenesis, has been decreased following LR and LC treatments. In addition, the expression of *SFRP2*, an antagonist of Wnt pathway, has been increased. Furthermore, we have demonstrated the downregulation of *ELF4E* expression following LC treatment. However, moderate decreases have been detected in expression of *HIF-AS1* following treatment with LC and expression of *SHARP1* as a result of LR treatments, after treated with LC, expression of *VHL* decreased that this result was unexpected.

Conclusions: *lactobacilli* can modulate expression of Wnt/  $\beta$ -catenin, mTOR and HIF-1 pathways genes in HeLa cell line. Between these three pathways Wnt/  $\beta$ -catenin, which is second hit in cervical cancer, showed the most change after treated with *lactobacilli*.

#### E-P12.034

##### Apoptotic Effects of Novel Benzimidazole Derivates On A549 Cell Line

G. Gulbul Duran<sup>1</sup>, N. Duran<sup>1</sup>, O. AlguP<sup>2</sup>, A. Yildirim<sup>1</sup>, M. Yaldiz<sup>1</sup>, A. Yoldas<sup>3</sup>;

<sup>1</sup>Mustafa Kemal University, Hatay, Turkey, <sup>2</sup>Mersin University, Mersin, Turkey,

<sup>3</sup>Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Turkey.

Background and Aim: Malignant cells are characterized by a number of alterations in controlling proliferation, differentiation, and apoptosis. New drug researches which inhibit the proliferation of cancer cells are quite important because of the drug resistance and side effect used in conventional cancer therapy. Benzimidazole derivates have been reported to be significant anti-proliferative activities. In this study we aimed to investigate the proliferative and anti/pro-apoptotic effects of new benzimidazole derivates on human lung adenocarcinoma epithelial cell line(A549).

Materials and Methods: Human Embryonic Kidney cells(HEK 293) and A549 cells were used in this study. A549 cells were treated with various concentrations of benzimidazole derivates. The expression levels of p53, Bcl-2, Bax, kaspaz-3 and NF-KB genes were analyzed in treated and control groups.

Results: In this study, the compounds was found to inhibit the cell growth at S phase. The compounds have been found to increase the expression of p53 gene. It was determined that the ratio of p53 expression increased in proportional to the incubation time. While, the compounds were found to be statistically significant reduction in the levels of antiapoptotic genes (bcl-2 and Bcl-XL), NF-KB level was found that the significantly increased. Also, caspase-3 activity in cancer cell line was observed significantly higher than compared to the control group ( $p<0,01$ ).

Conclusion: Benzimidazole derivatives compounds were found to be highly effective against human lung adenocarcinoma epithelial cells. These compounds found to be very promising compounds in the treatment of cancer therapy.

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#### E-P12.035

##### A Novel Translocation t(10;12)(q24;q13) In Childhood ALL

T. Akin Duman<sup>1</sup>, A. G. Zamani<sup>1</sup>, U. Çalışkan<sup>2</sup>, H. Tokgöz<sup>2</sup>, M. S. Yıldırım<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, Meram Medical Faculty,Necmettin Erbakan University, KONYA, Turkey, <sup>2</sup>Department of Pediatric Hematology, Meram Medical Faculty,Necmettin Erbakan University, KONYA, Turkey.

Acute lymphoblastic leukemia (ALL) is a malignant blood disorder characterized by clonal expansion of leukemic cells in the bone marrow, lymph nodes, thymus, or spleen. It is the most common childhood cancer. ALL accounts for 76% of among major types of leukemia and 43% of all deaths of pediatric leukemia patients in United States. Because of the diagnostic, prognostic and therapeutic value of the chromosomal findings, cytogenetic

analyses is important in ALL. Either in chromosome number (ploidy) or as structural changes as translocations, deletions and inversions are found in 90% of children with ALL. Translocations are the most common changes among structural abnormalities. In acute leukemias, structural abnormalities of chromosome 12 are well known. While the most frequently, the break occurs in the short arm, changes at the long arm are very rare.

A 12-year-old female patient had been diagnosed as ALL five years ago. She had chemotherapy along three years. 15 months after chemotherapy, she consulted at haematology with knee pain. Atypical blasts were shown in her control peripheral blood smear. Bone marrow aspirate examination was made and patient accepted as early isolated bone marrow relapse. Her bone marrow karyotype was as 46,XX[3]/46,XX,t(10;12)(q24;q13) [5] and 66% deletion on p16 gene was found in ALL FISH panel.

According to our knowledge, t(10;12)(q24;q13) is a novel translocation in childhood ALL. So that we report this case in order to contribute the literature.

#### E-P12.036

#### A novel cytogenetic aberration is extra isochromosome 4q in chronic lymphocytic leukemia patient

E. I. Atli<sup>1</sup>, H. Gürkan<sup>1</sup>, G. E. Pamuk<sup>2</sup>, C. Akurut<sup>1</sup>, H. Tozkur<sup>1</sup>, A. M. Demir<sup>3</sup>;

<sup>1</sup>Department of Medical Genetics, Faculty of Medicine, Trakya University, Edirne/Turkey, Edirne, Turkey, <sup>2</sup>Department of Haematology, Faculty of Medicine, Trakya University, Edirne, Turkey, <sup>3</sup>Department of Haematology, Faculty of Medicine, Trakya University, Edirne, Turkey.

Introduction: The genetic characterization of chronic lymphocytic leukemia (CLL) has made significant progress over the past few years. Chromosomal abnormalities are detected in up to 80% of patients.

Materials and Methods: Our patient was diagnosed with CLL stage 2 on 2012 and followed since then by hematology clinic. She is 63 years old. Bone marrow (BM) biopsy made and hypercellularity showing infiltration of atypical cells with CD5+, CD20+, CD23+ were determined. Hypoplasia are detected in myeloid/erythroid series and stage 2 reticular fibers proliferation were detected. Patient was followed up without medication. While follow-up of patients WBC:57300 HB:5,36 PLT:99700 are determined in may 2014. According to the patient's flow results CD5+, CD23+, FMC7+ were detected. Mature, small lymphocytes and smudge cell was found in the patient's peripheral blood smear. In USG imaging multiple lap was found in abdomen and multiple neck lymph nodes was detected. Patient BM aspiration were performed in 2014 and hypercellularity was found to contain 54% of atypical lymphocytes in the BM.

Results: Cytogenetic and FISH analysis is made from the patient's BM. According to the results of karyotyping and FISH 47, XX,+i4q is determined. According to literature, extra isochromosome 4q is reported by our case for the first time in CLL. She was diagnosed with stage 4 CLL and FCR treatment was initiated.

Conclusions: Our patient showed disease progression compared to previous results. So we offer that this evidence can be considered in terms of triggering the disease's progression or as a result of disease progression i4q was occurred.

#### E-P12.037

#### Impact of fluorescent in situ hybridization (FISH) aberrations and clu1 expression on the prognosis of chronic lymphocytic leukemia (CLL)

U. Abur<sup>1</sup>, G. Ogur<sup>1</sup>, O. Akar<sup>1</sup>, E. Altundag<sup>1</sup>, S. Aymelek<sup>1</sup>, M. Turgut<sup>2</sup>;

<sup>1</sup>Ondokuz Mayis University Medical Faculty Department Of Genetics, Samsun, Turkey,

<sup>2</sup>Ondokuz Mayis University Medical Faculty Department Of Hematology, Samsun, Turkey.

Introduction. The impact of genetic changes and CLLU1 expression in CLL patients were studied.

Materials-Methods. 156 patients were analyzed by FISH (LSI D13S139, LSI 13S25, LSI ATM, LSI P53, CEP12, IGH probes). FISH abnormalities were correlated with age, sex, WBC, Rai stage, survival, LDH, β2 microglobulin and treatment. CLLU1 expression was also quantified.

Results. FISH aberrations were found in 62% of patients: 13q14 deletion, 66.6%; trisomy 12, 27%; ATM deletion, 19%, TP53 deletion, 8% and IGH rearrangement 20%. Mortality rate was 40% with del 11q22 and del 17p (Tp53), 28% with del 13q14, 15% patients with trisomy 12. Overall survival was  $98 \pm 22$  mo for del 13q14, and  $69 \pm 19$  mo for del 11q22. Need of medication was 89% for del 11q22 and del 17p, 42% for others. The most frequent abnormality in progressive disease was ATM and TP53 deletion. Majority of the patients with stable disease showed heterozygous 13q14 deletion. Patients with homozygous 13q deletions yielded a shorter overall survival. Patients with IGH rearrangements yielded double increased mortality and treatment rate and shorter overall survival. There was no CLLU1 expression in healthy controls; in patients 1x 10<sup>-4</sup> CLLU1 expression was detected.

Higher CLLU1 expression was shorter survival and more patients needed medication (46% versus 21%).

CONCLUSION: Routine usage of FISH analysis is still indicated for prognostic evaluation of the CLL patients. Special consideration is needed for the poor prognostic implication of del 11q22, del 17p, IGH rearrangement and homozygous del 13q14. CLLU1 expression seems to have correlation with poor prognosis.

#### E-P12.038

#### Downregulation of apoptosis-related miRNAs in chronic lymphocytic leukemia

Z. Lasabová<sup>1,2</sup>, M. Vážan<sup>1</sup>, M. Grendar<sup>1</sup>, Z. Jedináková<sup>3</sup>;

<sup>1</sup>Comenius University in Bratislava, Jessenius Faculty of Medicine, Biomedical Center Martin, Martin, Slovakia, <sup>2</sup>Comenius University in Bratislava, Jessenius Faculty of Medicine, Dept. of Molecular Biology, Martin, Slovakia, <sup>3</sup>Comenius University in Bratislava, Jessenius Faculty of Medicine, Department of Hematology and Transfusiology, Martin, Slovakia.

Introduction: Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults with characteristic accumulation of monoclonal mature, functionally incompetent CD5+ lymphocytes co-expressing CD23 and CD19. We have focused on the deregulation of apoptosis -related micro RNAs (miRNAs) in CLL.

Material and methods: The microRNAs were extracted from peripheral blood of ten newly diagnosed patients with CLL without treatment and eight controls. There was designed a microplate with miRNAs which are documented to be involved in apoptosis in CLL - mir-222, mir-21, mir-143, mir-21 and mir-29, and miRNAs involved in apoptosis with an unknown role in CLL - mir-149, mir-200c, mir-204, mir-210, mir-221, mir-409, mir-449, let-7c, let-7g and mir-708. After stem-loop primer reverse transcription, the samples were analyzed using TaqMan chemistry and evaluated by delta-delta Ct algorithm with 7500 Software v2.0.1. Differences in relative gene expression were analyzed by Wilcoxon test of the mean RQ=1 at the significance levels p < 0.05.

Results: Application of the Wilcoxon test of the mean to test statistical significance for the differential miRNA expression showed that the downregulation is statistically significant for mir-133b, mir-143, mir-149, mir-200c, mir-204, mir-221, mir-222, mir-409 and mir-708.

Conclusion: The downregulation of miRNAs expression can be caused by their promoter methylation which was already demonstrated for mir-708 in CLL. We are going to validate these results on the larger patient's cohort and to analyze the role of miRNA methylation in the downregulation of these miRNAs.

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#### E-P12.039

#### Aurora Kinase Inhibitor CCT137690 can Induce Apoptosis on Chronic Myeloid Leukemia Cells

A. Asik, C. Kayabasi, B. Ozmen Yelken, T. Balci, S. Gonca, F. Sogutlu, R. Gasimli, S. Yilmaz Susluer, C. Biray Avci, C. Gunduz, Ege University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkey.

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease resulting from neoplastic transformation of multipotent stem cells. It is characterized by the Philadelphia chromosome (Ph) occurred in 95% of cases, resulting from translocation t(9;22)(q34;q11.2) which leads to the formation of Bcr-Abl fusion gene encoding a product involved in CML pathogenesis. Aurora kinases are the most important serine/threonine protein kinases regulating function of centrosomes, spindles and kinetochores. It has been reported that aurora A and B have overexpression in various malignancy such as breast, colon, neuroblastoma, pancreas and over cancer. However, it has been indicated that Bcr-Abl induce the expression of these kinases in CML cells and claim that aurora kinase inhibitors have potential advantage in treatment of CML. CCT137690 is a highly selective aurora kinase inhibitor. In this study, we aimed to determine the apoptotic effect of CCT137690 on KU812 human chronic myeloid leukemia cells.

WST-8 assay was done to determine cytotoxic effect of CCT137690 on KU812 cells and IC50 dose was found 6.24 μM for 48 h. The effect of IC50 dose on apoptosis and cell cycle was evaluated with Annexin V-EGFP Apoptosis Detection Kit and BD Cycletes Plus DNA Reagent Kit, respectively. The IC50 dose CCT137690 was found to induce apoptosis in 9-fold compared to control and to arrest cell cycle at G2/M phase.

In conclusion, this study has supportive quality for opinion that aurora kinase inhibitors have potential advantage in treatment of CML, because of that CCT137690 highly induce apoptosis and cause cell cycle arrest.

## E-P12.042

**BCR-ABL1 positive chronic myeloid leukemias with cryptic rearrangements without Philadelphia chromosome: two case reports**  
*C. Correia<sup>1,2</sup>, S. Lisboa<sup>1</sup>, S. Bizarro<sup>1</sup>, M. J. Oliveira<sup>2</sup>, N. Salgueiro<sup>2</sup>, L. Torres<sup>1</sup>, J. Vieira<sup>1</sup>, N. Cerveira<sup>1</sup>, S. Castedo<sup>2</sup>, M. R. Teixeira<sup>1</sup>;  
<sup>1</sup>Portuguese Oncology Institute, Porto, Portugal, <sup>2</sup>GDPN, LABCO, Porto, Portugal.*

**Introduction:** Chronic myeloid leukaemias (CML) usually harbour a reciprocal chromosomal translocation t(9;22) that results in the Philadelphia chromosome, leading to the formation of the *BCR-ABL1* fusion gene. In a minority of CML patients, the *BCR-ABL1* chimera can be formed through cytogenetically cryptic rearrangements and present an apparently normal karyotype. In order to correctly identify all the patients that can benefit from a TKI therapy, a multidisciplinary approach should be taken.

**Materials and methods:** Classical cytogenetics, molecular studies and fluorescence in situ hybridization (FISH) were performed in bone marrow samples of two CML cases.

**Results:** Karyotype analysis of both cases did not show chromosomes 9 and 22 rearrangements. Molecular analysis revealed the presence of the *BCR-ABL1* fusion gene. FISH studies showed the occurrence of the *BCR-ABL1* fusion in chromosome 9 in both cases.

**Conclusion:** These two examples demonstrate the importance of a multidisciplinary approach to establish a correct genetic diagnosis and characterization of CML cases.

## E-P12.043

**The evaluation of the effects of dasatinib on protein expression and the enzymatic activity of PP2A catalytic and regulatory subunits that are dasatinib-treated K652 cell line**

*B. Özel<sup>1</sup>, S. Kıpçak<sup>1</sup>, Ç. Aktan<sup>1</sup>, Ç. Biray Avcı<sup>1</sup>, C. Gündüz<sup>1</sup>, G. Saydam<sup>2</sup>, N. Selvi Günel<sup>1</sup>;  
<sup>1</sup>Ege University Department of Medical Biology, Izmir, Turkey, <sup>2</sup>Ege University Department of Hematology, Izmir, Turkey.*

**Introduction:** Chronic myeloid leukemia (CML) is characterized by increased tyrosine kinase activity due to BCR/ABL translocation. Dasatinib is tyrosine kinase inhibitor that is more effective than imatinib but 20% of patients don't respond to treatment. Therefore, other molecular approaches should be investigated. For that purpose, protein phosphatase 2A (PP2A) that is found genetically altered or functionally inactive in CML, may be promising therapeutic target. PP2A is consist of three subunits and involved in many cellular function. In our study, we aimed to evaluate PP2A enzyme activity and protein level in dasatinib treated K562 cell line.

**Materials and Methods:** The cytotoxic effect of dasatinib was evaluated by WST-1 analysis. Apoptosis was determined by Annexin V and Apo-Direct Tunel assays. Okadaic acid (OA) was used as PP2A inhibitor. The changes of PP2A enzyme and protein levels were observed by serine/threonine phosphatase and western blot analysis, respectively.

**Results:** The cytotoxic effect of dasatinib in K562 cells was found 4.6 nM at 48th hour. According to Annexin V, apoptosis was increased 2.35 and 3.76 fold by treating with dasatinib and 2.5  $\mu$ M and 25  $\mu$ M OA combination, respectively. According to Tunel assay, apoptosis was induced 16.45 and 49.38 fold in same order. The PP2A enzyme activity decreased compared to control at 72nd hour. Protein level of PP2A catalytic subunit decreased compared to control by dasatinib treatment.

**Conclusions:** We found that PP2A has major role in cell homeostasis and apoptosis. Thus, we could say that targeting PP2A inhibitors may be promising therapeutic approach for CML.

## E-P12.046

**Detection of KRAS codon 12 and 13 mutations by Allele-specific polymerase chain reaction assay**

*N. Chaichanawongsaroj;  
 Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.*

**Introduction:** Mutations of *KRAS* gene at codon 12 and 13 are resistant to targeted therapies with anti-epidermal growth factor receptor (EGFR) antibodies in colorectal cancer. Detection of *KRAS* mutations is critical for effective and appropriate treatment for individual patients. The aim of the present study was to develop Allele-Specific PCR (AS-PCR) assay for analysis of the mutational status of *KRAS* codons 12 and 13, at nucleotides 34, 35 and 38.

**Materials and Methods:** DNA was extracted from 41 formalin-fixed paraffin-embedded (FFPE) colorectal cancer tissues. The AS-primers for specific amplifying wild-type *KRAS* were established. The assay has been validated with pyrosequencing method. The sensitivity and limit of detection were determined.

**Results:** Three AS-primers were successfully distinguished wild-type *KRAS*

from the mutant alleles at nucleotides 34, 35 and 38. The limit of detection was detected at 50-55% mutant allele with the lowest amount of DNA at 10 ng/ul. The results of AS-PCR assay were in good concordance with pyrosequencing only in samples with % mutant alleles greater than 60%. The sensitivity and specificity of our AS-PCR were 43.33% and 100%, respectively. Conclusions: We demonstrated that AS-PCR specific for wild-type *KRAS* at nucleotides 34, 35 and 38 could detect mutations in highly mutant enriched tumor samples.

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## E-P12.047

**Therapeutic potential of post-transcription MCM4 gene silencing for colorectal cancer treatment**

*A. Kuzevanova, A. Koroteva, A. Alimov, A. Karpukhin;  
 Research Centre of Medical Genetics, Moscow, Russian Federation.*

**Introduction:** The RNAi based approaches for cancer treatment implies searching for new targets genes. The MCM4 gene encoding the subunit of helicase, is involved into development replication stress and cell cycle control. The purpose of this study to increase knowledge on MCM4 gene expression, evaluate the possibility of its use in tumor therapy.

**Materials and Methods:** The study conducted using colorectal cancer cells line HT-29. Lipofectamine RNAiMAX was used for delivery of designed siRNA. Flow cytometry was employed for cell cycle analysis. Antibodies against AIF protein and fluorescent inhibitor of caspases-3 and -7 used for apoptosis study.

**Results:** It was discovered that low dose of oxaliplatin (1-5mM) up-regulates MCM4 gene mRNA expression more than 3-5 folds. The gene silencing induces apoptotic HT-29 cell death. The event is time- and dose-dependent. It was shown that MCM4 silencing lead to accumulation of cells in G1 phase after 48 hours of exposure. Apoptotic cell death reached 60%. The analysis of apoptotic pathways revealed that there is no influence of AIF. The main mechanism of cell death is associated with identified activation of caspases -3 and -7. Adding to the cell culture inhibitor of caspases-3 and -7 (Z-DEVD-FMK) significantly reduces the number of cells undergoing apoptosis.

**Conclusion:** Thus, the activation of caspase-dependent pathway is the main cause of cell death after MCM4 gene silencing in presence of oxaliplatin. This opens up new opportunities to create target drugs, which should help to avoid the problem of resistance during oxaliplatin treatment.

## E-P12.048

**Frequency of chromosomal abnormalities in patients with Multiple Myeloma**

*C. Aydin Acar<sup>1</sup>, C. Hangu<sup>2</sup>, O. Salim<sup>3</sup>, O. K. Yucel<sup>3</sup>, S. Berker Karauzum<sup>2</sup>;*

*<sup>1</sup>Mehmet Akif Ersoy University, Bucak School of Health, Burdur, Turkey, <sup>2</sup>Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, Antalya, Turkey, <sup>3</sup>Akdeniz University, Faculty of Medicine, Department of Hematology, Antalya, Turkey.*

An uncontrolled proliferation of plasma cells which play a role in immune system is called Multiple Myeloma (MM). It is the second most common hematologic malignancy in the United States, and accounts for approximately 1% of all cancer cases. Currently, detection of chromosomal abnormalities is accepted as a important tool for predicting outcome in newly diagnosed MM patients (1). In this retrospective study, we aimed to determine cytogenetic findings using by conventional cytogenetics and FISH methods in 435 patients with MM. Cytogenetics analysis was successful in 367 patients (84,4%) and unsuccessful in 68 patients (15,6%). Ninety nine of 367 (27%) patients were found to have an abnormal karyotype. Of 99 patients, 19 patients (19,2%) had hyperdiploidy (>47), 40 patients (40,4%) had hypodiploidy ( $\leq 45$ ), and 25 patients (25,2%) had pseudodiploidy. In addition, only the loss of Y chromosome was found in 15 patients (%15,2) and these patients were classified in as a separate group. The presence of both structural and numerical chromosome abnormalities were detected in 36 patients (36,4%); 40 patients (40,4%) had only numerical abnormalities; 23 patients (23,2%) had only structural abnormalities. In this study, specific FISH probes for t(4;14), t(11;14), t(14;16), del(11q22.3), del(17p13) and del(13q14) was applied to the slides of patients. By FISH analysis, the most frequently abnormality, del(13q14), was observed in 14,5% (33/228) and del(17p) was determined the second most frequently abnormality in 7,65% (18/235) of patients.

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**E-P12.050****MYC rearrangements in diffuse large B cell lymphoma patients**

**B. Durak Aras<sup>1</sup>, S. Isik<sup>1</sup>, O. Cilingir<sup>1</sup>, H. Uskudar Teke<sup>2</sup>, M. Ozdemir<sup>1</sup>, E. Gündüz<sup>2</sup>, H. Aslan<sup>1</sup>, S. Artan<sup>1</sup>, M. O. Akay<sup>1</sup>;**

<sup>1</sup>Eskisehir Osmangazi University Medical Faculty Department of Medical Genetics, Eskisehir, Turkey, <sup>2</sup>Eskisehir Osmangazi University Medical Faculty Department of Hematology, Eskisehir, Turkey.

Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease with its clinical, immunohistochemical, morphological and molecular features. MYC rearrangements occur in 5-10% of DLBCL patients. Since we have very limited experience in patients with isolated MYC rearrangements, the prognostic significance of this cytogenetic abnormality is still unknown.

**AIM:** In this study, we aimed to investigate the frequency and prognostic significance of MYC rearrangements in patients with DLBCL.

**MATERIAL AND METHODS:** A total of 46 patients with DLBCL were included in this study. Deparaffinized samples were evaluated by FISH using MYC, BCL6 and BCL2/IGH and CCND1/IGH probes.

**RESULTS:** Rearrangement of MYC was found in 16 patients (34%). Of these patients, 9 were double-hit and 2 were triple hit lymphomas. When we compared the prognostic markers including stage, IPI and LDH between MYC+ and MYC- patients, we found a significant difference in stage and LDH values between the groups ( $p=0.004$  and  $p=0.037$ , respectively). IPI score did not show any difference between isolated MYC+, two hit and three hit patients ( $p>0.05$ ).

**DISCUSSION:** MYC rearrangements confer a worse effect on prognostic factors such as IPI, tumor stage, and survival. In this study, tumor stage and LDH values showed significant difference between MYC+ and MYC- patients in consistent with the previous literature. However, the presence of BCL2 and BCL6 rearrangements in MYC- patients conferred a prognostic effect. To address the prognostic impact of MYC rearrangement, we need a higher number of isolated MYC+ and three negative patients considering BCL2 and BCL6 aberrations.

**E-P12.051****The effects of a novel resveratrol analog known as DMU-212 on leukemia stem cell line**

**B. Ozmen Yelken, C. Kayabasi, T. Balcı, A. Asik, S. Gonca, F. Sogutlu, R. Gasimli, S. Yilmaz Susluer, C. Biray Avci, C. Gunduz;**  
Ege University Medical School, Department of Medical Biology, Izmir, Turkey.

Myeloid leukemias results from malignant transformation of primitive hematopoietic cell and it is suggested that leukemia stem cells are malignant derivatives of normal hematopoietic stem cells. Leukemia stem cells have similar features with normal stem cells including self-renewal, differentiation and infinite proliferation. Resveratrol, which is a potential chemopreventive and chemotherapeutic agent, is found in red grape. Previous studies have shown that resveratrol has anti-tumorigenic effect in several cancer types. It has been shown that DMU-212 usually induces G2/M arrest through limited studies which are related to resveratrol analogs.

In our study, we aimed to evaluate the effects of DMU-212 on apoptosis, cell cycle and also proliferation in human leukemia stem cells.

Cytotoxicity of DMU-212 (30  $\mu$ M - 0.5  $\mu$ M) on human leukemia stem cell line (LSC) were evaluated with Cell Proliferation Reagent WST1 test. Changes of apoptosis and cell cycle in LSCs with exposure to IC50 dose of DMU-212 are detected with AnnexinV-EGFP Apoptosis Detection Kit and Cycletest Plus DNA Reagent Kit with FACS, respectively.

IC50 dose of DMU-212 was calculated as 27,97  $\mu$ M. It was shown that DMU-212 couldn't induce apoptosis during 48 hours. Beside that, LSCs accumulated in G2/M phase of the cell cycle with the exposure of 27,97  $\mu$ M DMU-212. In conclusion, our hypothesis suggests that DMU-212 may influence the cell cycle pattern of leukemia stem cell line due to G2/M arrest significantly. Therefore, DMU-212 may be considered as an additional anticancer agent in leukemia treatment.

**E-P12.053****High Content and High Throughput Single Cell mRNA Sequencing Analysis of Prostate Cancer Cells Using Fluorescence Activated Flow Cytometry Combined with Molecular Barcode Technology**

**M. A. Tycon;**  
Becton Dickinson, San Jose, CA, United States.

EpCAM, a surface adhesion protein implicated in cancerous cells gaining metastatic potential, is dysregulated, exhibiting varied expression among prostate cancer samples and cell lines. Prostate cancer cell lines PC3, DU145, and LNCaP exhibit variable EpCAM expression. While the metastatic potential of each line is understood, there is little consensus on EpCAM ex-

pression. Using single cell mRNA sequencing enables simultaneous observation of hundreds of genes, elucidating the interplay of EpCAM in metastasis. The BD FACSseq™, a new easy-to-use sorter, was employed to deliver single cells and eliminate doublets or higher order aggregates for single cell mRNA-Seq analysis. Accurate single cell delivery into 96-well plates was confirmed. Several hundred cells were examined by transcript analysis of 110 genes associated with prostate cancer using BD™ Precise encoded plates, which incorporate molecular indexing and sample barcodes to achieve high-fidelity gene profiling. For each of the three cell lines, EpCAM protein level distribution was recorded through index sorting, revealing broad distributions for PC3 and DU145. Index sorting on the BD FACSseq enabled protein expression to be tied to gene transcript count for each cell analyzed. Genes expressing differential regulation from the panel were correlated to EpCAM expression, indicating which may influence metastatic potential. The accurate delivery of individual cells by flow cytometry paired with single cell sequencing technologies can enable researchers to investigate transcriptional dynamics at the individual cellular level, disambiguating sample heterogeneities. These results aid in understanding the centrality of EpCAM, a possible therapeutic target, during the transition to androgen resistant, metastatic cancers.

**E-P12.055****The investigation of APC mutations in Russian patients with Familial Adenomatous Polyposis**

**A. S. Tsukanov, V. Shubin, N. Pospekhova, I. Sachkov, A. Kuzminov, V. Kashnikov, Y. Shelygin;**

Ministry of Health / State Scientific Centre of Coloproctology, Moscow, Russian Federation.

Familial Adenomatous Polyposis (FAP) is responsible for 1% of colorectal cancer cases. FAP is caused by germline mutations in APC gene (mutations frequency varies between 40-75% in different populations). The aim of this investigation was to study the frequency of APC mutations in the Russian cohort of FAP patients.

We collected blood samples of 107 patients under 45 years with the presence of > 100 colorectal polyps. We also studied blood samples of 35 patient family members. Germline mutations in APC gene were analyzed by PCR, electrophoresis, Sanger Sequencing and Next-Generation Sequencing. Germline mutations in APC were detected in 77 from 107 patients (72%). Among these 77 mutations met recurring: p.Arg232X (2 cases), p.Asp849GlufsX11 (2 cases) p.Arg216X (3 cases), p.Gln1062X (4 cases), p.Arg213X (5 cases), p.Glu1309AspfsX4 (16 cases). From 51 unique mutations 21 were nonsense, 25 - frameshift and 5 - splicing site mutations. Twenty-two mutations were described for the first time. Mutations in APC are located throughout the gene from 142 to 1492 codons, while 25 from 51 variants - in codons 900-1500. In 16 from 35 relatives germline mutations in APC were found, what led to their inclusion into the risk group. The frequency of APC mutations in Russian patients with FAP was 72%. Twenty-two mutations were described for the first time.

**E-P12.057****Peculiarities of gene expression in disseminated gastric cancer**

**F. Kipkeeva<sup>1</sup>, T. Muzaffarova<sup>1</sup>, A. Karpukhin<sup>1</sup>, M. Narimanov<sup>2</sup>, O. Malekhova<sup>2</sup>;**

<sup>1</sup>Research Centre for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>NN Blokhin Russian Cancer Research Centre, Moscow, Russian Federation.

Patients with disseminated gastric cancer have a poor prognosis. The study of gene expression is important for understanding of tumor progression and for developing predictive and prognostic markers. We studied among patients with disseminated gastric cancer the expression profiles of the genes - potential markers (VEGF, VEGFR1, VEGFR2, NRP-1, bFGF, FGFR2, TGF- $\beta$ , HER2/neu, TUBB, BRCA1, Ki67, PCNA) - by quantitative real-time PCR in paired tumor - normal tissue samples. Correlations in the studied gene expression levels were also determined.

An objective response to therapy with Herceptin was observed under increased expression of HER2 gene. Tubulin gene expression was associated with the effective action of taxanes ( $p = 0.036$ ). The FGFR2 gene is under consideration as a new therapeutic target. However, the incidence of increased expression of FGFR2 was only 5%, which corresponds to the studied elsewhere its frequency amplification at gastric cancer.

Most often elevated mRNA level in the tumor relative to the control was observed for NRP-1, VEGFR2 and TGF- $\beta$  genes (32% - 41%). The correlation of these gene expression levels was found for the first time ( $R=0.63$ ;  $p=0.002$ ). Survival more than 1 year was associated with simultaneously increased expression level of these genes ( $OR=10.6$ ;  $95\%CI = 1.48$  to  $76.08$ ;  $p=0.018$ ). An inverse correlation VEGF and bFGF gene expression was revealed also ( $R=$

0.73;  $p=0.005$ ). This effect probably reflects alternative ways to stimulate the gastric cancer development and must be taken into account in the choice of means of targeted therapy for this disease.

#### E-P12.059

##### The mRNA expression of PHD1 gene of chronic myeloid leukemia cells

A. Toylu, O. Salim, O. Altioğlu Clark, I. Undar;  
Akdeniz University, Antalya, Turkey.

PHD1 gene is a hydroxylase enzyme regulating the proteosomal degradation of Hif1a transcription factor which is related with the expression of several genes responsible for the cancer development and cancer cell survival. It has been reported that PHD1 protein also regulates the expression of some other proteins related with malign cellular behavior but the expression level of PHD1 gene was remain mostly unclear. Recent studies on bone marrow stem cells showed that PHD inhibitors stimulate the hematopoietic stem cell (HSC) mobilization. Furthermore, PHD2 gene ablation was reported to increase the self-renewal of HSCs. To better understand the role of PHD1 gene on malign hematopoietic cells we performed the expression analysis of PHD1 gene of chronic myeloid leukemia (CML) cells. Patients were collected into; at the diagnosis stage, tyrosine-kinase inhibitor therapy responsive- or non-responsive groups. Peripheral blood and bone marrow samples of CML patients were obtained to investigate mRNA expression of PHD1 gene. ACTB gene was used as a reference gene. Real time quantitative PCR and REST analysis were performed to measure expression levels. PHD1 gene expression was higher in the therapy responsive group than both the diagnosis stage and the therapy non responsive groups. According to our data, the low level of PHD1 gene expression at the CML diagnosis or the therapy resistant states is most likely related with the high levels of cell survival regulatory genes. The relationship between PHD1 protein and its substrate proteins needs to be further analyzed in CML.

#### E-P12.060

##### Differential expression of genes involved in biotransformation mechanism in oral cancer

A. Russo<sup>1</sup>, P. M. Biselli-Chicote<sup>1</sup>, W. Chaves<sup>1</sup>, M. M. U. Castanhole-Nunes<sup>1</sup>, C. R. S. Silva<sup>1</sup>, A. P. A. Gimenez-Martins<sup>1</sup>, T. Z. Lopes<sup>1</sup>, J. V. Maniglia<sup>2</sup>, D. Santi-Neto<sup>2</sup>, É. C. Pavarino<sup>1</sup>, E. M. Goloni-Bertollo<sup>1</sup>;

<sup>1</sup>Research Unit in Genetics and Molecular Biology – UPGEM / São José do Rio Preto Medical School (FAMERP), São José do Rio Preto, Brazil, <sup>2</sup>Department of Otorhinolaryngology and Head and Neck Surgery – FAMERP, São José do Rio Preto, Brazil.

Introduction: Differential expression of genes that encoded enzymes involved in the biotransformation mechanism of endogenous and exogenous compounds by oxidation reactions, as cytochrome P450 (CYP) family and others oxygenases enzymes, can change the activation process of toxic agents and lead to oral tumor development.

Materials and Methods: Eight tumor samples and eight adjacent non-tumor tissue samples of patients with oral cavity squamous cell carcinoma were included in this study. TaqMan® Array Human CYP450 and other Oxygenases 96-well fast plate (Applied Biosystems) was used to evaluate the gene expression pattern in oral cavity tumors by real time qPCR. For statistical analysis was performed D'Agostino & Pearson omnibus normality test, followed by One-sample T test or Wilcoxon signed rank test using GraphPad Prism v.5 program. Correction for multiple testing of Benjamini-Hochberg False Discovery Rate was applied.

Results: CYP27B1 gene was overexpressed and CYP27A1, CYP2E1, CYP2R1, CYP2J2, CYP2U1, CYP4F12, CYP4X1, PTGIS, ALOX12, CYP4B1 and MAOB were down expressed in oral squamous cell carcinoma tissue ( $p<0.05$ ). After correction by multiple tests, PTGIS gene remained differentially expressed.

Bioinformatics analyses showed that CYP2E1, CYP2J2, CYP2U1, ALOX12 and PTGIS proteins were involved in the arachidonic acid metabolism associated with important inflammatory processes in carcinogenesis.

Conclusion: Genes involved in the oxidation reactions showed differential expression in oral squamous cell carcinoma. The enzymes encoded by these genes play an important role in the arachidonic acid metabolism, which can influence the regulation of important physiological mechanisms in oral tumorigenesis process.

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#### E-P12.061

##### Sensitivity of a human glioblastoma cell line U251 to all-trans retinoic acid treatment

D. Drakulic, J. Marjanovic Vicentic, N. Kovacevic Grujicic, M. Schwirtlich, M. Stevanovic; Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia.

Introduction: Gliomas are the most common primary brain tumors in humans. Glioblastoma, grade IV of glioma tumors, is one of the most aggressive and deadly forms of cancer with the median survival of 15 months even though significant advances in treatment strategies. Retinoic acid (RA), an active metabolite of vitamin A, modulates the proliferation, differentiation and apoptosis in a wide variety of normal and tumor cells. It has been used in clinical trials on glioma tumors, but the efficiency of treatment was heterogeneous.

Materials and Methods: U251 cell line, one of the most widely used *in vitro* model system for studying glioblastoma pathobiology, was used to analyze the effects of RA on cell morphology (staining of microtubule cytoskeleton protein), viability (MTT test), proliferation (Ki-67 staining), migratory potential (Wound Scratch Assay Test) and adhesion ability (Cell-Matrix Adhesion Assay). Cells were exposed to 10, 20 and 40 microM all-trans retinoic acid (ATRA) and analyzed in two time points (3 and 5 days).

Results: Our results reveal that ATRA can cause changes in cell morphology, viability and migratory capabilities. On the other side, treatment with ATRA did not influence the U251 proliferation rate. Additionally, only 5-days treatment of U251 cells with 10microM ATRA changes the adhesion ability of U251 cells compared to control cells.

Conclusions: Obtain results indicate that ATRA treatment is associated with modification of some important features of U251 cells.

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#### E-P12.062

##### Fucoidan from *Fucus vesiculosus* inhibits proliferation and upregulates p21 level in UT-SCC-74A head and cancer cell line

E. Gunduz, K. Akbas, S. Ciftci Yilmaz, O. Hatipoglu, H. Oksuz, A. Bayatoglu, R. Bayrak, M. Gunduz; Turgut Ozal University, Ankara, Turkey.

Head and neck cancer accounts for about 15% of all cancers, the great majority of which are of the squamous cell type and include malignant tumors of the larynx, pharynx, mouth and nasal cavity. The treatment of Head and Neck Cancer is difficult from other types of cancer if it is not early diagnosed. Thus, pursuit of finding alternative treatment methods directed scientists to investigate the potential plant and chemical sources.

Mozuku, type of moss/algae, is a commonly consumed food in Japan and is a rich source of alginic acid, carotenoid, vitamin C, vitamin K, amin acids, iron, phosphorous, calcium and fucoidan. Fucoidan, a complex sulphated polysaccharide, has been reported to have several biological activities including anti-inflammatory, anti-angiogenic and anti-tumor activities. In our presented research, we investigated the anti-tumor activity of Fucoidan on the UT-SCC-74A head and neck cancer line.

First, we investigated the anti-proliferation activity of fucoidan by using xCELLigence Real Time Cell Analysis system. Fucoidan inhibits the cancer cell proliferation compared to the control group. Afterwards, to investigate whether this inhibition was due to cell cycle arrest, we investigated the p21 level. p21 level was significantly upregulated in the cancer cell line after fucoidan treatment. Even though our preliminary result suggest that inhibition of proliferation observed was potentially due to cell cycle arrest, further experiments needed to confirm and support our findings. Nevertheless, based on our results, Fucoidan has a great potential in the head and neck cancer treatment.

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#### E-P12.065

##### Evaluation of Genetic Mutations in BRCA1, BRCA2 and CHEK 2 Genes as Risk Factors for the Development of Breast and Ovarian Cancer

F. A. Pinarli<sup>1</sup>, K. M. Erdogan<sup>2</sup>, M. Y. Alp<sup>1</sup>, M. K. Altundag<sup>3</sup>, Ö. Dizdar<sup>3</sup>, H. E. Tekin<sup>4</sup>, A. Yesilyurt<sup>1</sup>;

<sup>1</sup>Dışkapi Yıldırım Beyazıt Research and Training Hospital Genetic Diagnostic Center, Ankara, Turkey, <sup>2</sup>Etilik Zubeyde Hanım Gynecology and Obstetrics Training and Research Hospital, Department of Medical Genetics, Ankara, Turkey, <sup>3</sup>Hacettepe University Faculty of Medicine, Medical Oncology, Ankara, Turkey, <sup>4</sup>Gazi University Faculty of Medicine, Medical Oncology, Ankara, Turkey.

Mutations in the BRCA1, BRCA2 and CHEK 2 genes are well-established risk factors for the development of breast and ovarian cancer. There is few studies about the spectrum and frequency of mutations in these genes in the

Turkish Population. After DNA isolation from blood samples of patients presented to our clinic with a diagnosis of familial and/or early-onset breast or ovarian cancer, BRCA1 and 2 genes were analyzed with both Sanger sequencing and MLPA, while CHEK 2 gene analysis was only performed with MLPA. A total of 169 patients with 125 index cases and 44 family members were included in the study. There were 10 males and 172 females, including 111 patients with breast cancer, 11 patients with ovarian cancer, 3 patients with both breast and ovarian cancer, and 44 relatives. Mutations were found in only 23 patients among index cases: BRCA 1, BRCA2, and CHEK2 mutations were found in 15, 7, and 1 patients, respectively. Mutations were detected with DNA sequence analysis in 20 cases and with MLPA in 3 cases. Although there are genetic markers identifying women and men with an increased risk for breast and ovarian cancers, the majority of inherited risk factors remains undetermined. Mutations in the BRCA1 and BRCA2 genes confer a substantial increase in breast cancer risk but routine clinical genetic screening is limited to the coding regions and intron/exon boundaries. Nowadays, it will be more informative and cost effective if all genes related with breast and ovarian cancer can be analyzed together.

#### E-P12.066

##### Identification of a novel variant in the MSH2 translation initiation codon

I. Wunderlich, A. Holtorf, T. Hinrichsen, O. Wachter, I. Rost;

Zentrum für Humangenetik und Laboratoriumsdiagnostik, 82152 Martinsried, Germany.

HNPCC/Lynch syndrome (LS) is one of the most frequent cancer susceptibility syndromes causing an increased risk for several solid tumors, including gastrointestinal and endometrial cancers. HNPCC/LS is caused by germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2, with MSH2 representing the second most frequent affected gene. Here we report the case of a 67 year old female with metachronous colorectal cancer without any family history of cancer. The attending physician ordered pathological analysis of tumour tissue to identify microsatellite status and immunohistochemical (IHC) status of the MMR proteins. The tests revealed highly microsatellite instability and inconsistent IHC results due to lack of both MSH2 and PMS2 proteins. Subsequent sequencing of all four MMR genes via Next Generation Sequencing and Sanger technology, as well as MLPA analysis of MLH1, MSH2, MSH6 and PMS2 was performed to identify small and larger sequence variants. We detected the heterozygous variant c.2T>G in MSH2 which affects the translation initiation codon of the gene. The variant is predicted to lack 25 amino acids at the N-terminus of the protein. Without any family history of HNPCC-associated cancers, and the cancer diagnosis made at the patient's age of 61, the phenotype appears to be not severe. Nevertheless, because tumour DNA showed highly microsatellite instability, together with *in silico* data this variant is supposed to reduce MSH2 protein activity and might be considered as likely pathogenic.

#### E-P12.067

##### Analysis of Ki67 and several clinical-pathological variants in Mexican patients with breast cancer

L. Plaza-Benumea<sup>1</sup>, M. Martin de Soto<sup>2</sup>, M. Hernandez-Gonzalez<sup>3</sup>, S. Cuevas-Covarrubias<sup>4</sup>;

<sup>1</sup>Departamento Genética Hospital para el Niño Toluca, IMIEM, Edomex, Mexico, <sup>2</sup>Departamento Genética Hospital Materno Infantil, ISSEMYM, Edomex, Mexico, <sup>3</sup>Patología, Facultad de Medicina, Hospital General de México, Universidad Nacional Autónoma de México, Mexico City, Mexico, <sup>4</sup>Genética, Facultad de Medicina, Hospital General de México, Universidad Nacional Autónoma de México, Mexico City, Mexico.

Introduction: Breast cancer is the most common malignancy in women, with more than 1.4 million cases diagnosed each year worldwide. Ki67 is a cell proliferation marker whose expression in breast tumors has been associated with poor prognosis. The aim of this study is to evaluate the relationship between Ki67 and several clinical-pathological factors. Methods: 416 cases with breast cancer of the General Hospital of Mexico were included. Results: The most common histological type was ductal (84.1%) with a size under 2 cm (55.8%), mean-age at diagnosis was 50.78, the most frequent tumor marker was luminal B Her2+ (24.5%); Ki67 was positive in 70.9% of all tumors. Significant association was observed in Ki67 with positive familial history of cancer (p 0.002), parity (p 0.002), breastfeeding (p 0.002), postmenopausal (p 0.018), and IIIA TNM stage (p=0.003). Ki67 was found more frequently in HER2+ tumors (p <0.001). Ki67 is mainly found in Luminal B HER2+ and in triple negative, in both cases Ki67 is associated with poor prognosis (P <.001) and with a great number of relapses (P <.001). Conclusions: Our results indicate that there is a significant association between Ki67 and histological grade, tumor size, presence of HER2. We also detected the worse prognosis in breast cancer triple negative with high levels of Ki67. Grant DGAPA/PAPIIT/UNAM Project IN204114-2.

#### E-P12.068

##### Gene variants in miRNAs binding site and chronic myeloid leukemia risk

H. Gutiérrez-Malacat<sup>1</sup>, M. Ayala-Sánchez<sup>2</sup>, J. Domínguez-Rodríguez<sup>2</sup>, X. Aquino-Ortega<sup>2</sup>, L. Orozco<sup>1</sup>, E. J. Cordova<sup>1</sup>;

<sup>1</sup>National Institute of Genomic Medicine, Mexico City, Mexico, <sup>2</sup>Hospital of Specialities, CMN La Raza, IMSS, Mexico City, Mexico.

Introduction: Chronic myeloid leukaemia (CML) is a malignant hematopoietic disease, characterized by the presence of the BCR-ABL fusion gene. On Spite of its clinical importance, little is known about the genetic factors associated with CML development. The miRNAs are small non-coding RNAs that regulate gene expression by binding to the 3' UTR of the target mRNA. Gene variants altering miRNA:mRNA interaction have been described as a risk factor in cancer. The aim of this study was to evaluate the association of variants that alter miRNA:mRNA interaction with CML risk.

Materials and Methods: Identification of variants located at miRNA binding site in coding genes was performed by using public databases and bioinformatics tools. From the identified variants, 384 were genotyped in 200 CML individuals and 200 healthy donors by using custom microarrays. The top associated variants with CML were validated in 300 CML subjects and 500 healthy donors by using TaqMan probes.

Results: We identified over 59,000 SNPs in 3'UTR sequences. From the 384 variants chose for the case-control study, the SNPs rs11680458, rs11638, rs6218, rs2422976, rs6728684, rs3025053, rs1799782, rs11211037, rs4987843 and rs12194974 showed a significant difference in their allelic distribution among cases and controls. However, after increasing sample size only the rs11680458 and rs11638 SNPs, located in the genes WDR43 y HELLS, respectively showed a significant association with an increased risk of developing CML.

Conclusions: The rs11680458 and rs11638 SNPs located in the genes WDR43 y HELLS could be associated with the susceptibility to CML.

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#### E-P12.070

##### Detection of EGFR mutations and ALK rearrangements in Non-Small-Cell Lung Cancer (NSCLC) Romanian patients for TKI targeted therapy suitability

P. Apostol<sup>1</sup>, R. Motoc<sup>1</sup>, F. Jordache<sup>1</sup>, P. Gurban<sup>1</sup>, G. Dinulescu<sup>1</sup>, P. Iorga<sup>2</sup>, A. Ungureanu<sup>2</sup>;

<sup>1</sup>Personal Genetics - Medical Genetics Center, BUCHAREST, Romania, <sup>2</sup>University Emergency Hospital of Bucharest, BUCHAREST, Romania, <sup>3</sup>Radiotherapy Center Cluj, Cluj-Napoca, Romania.

In the last years, the use of tyrosin-kinase inhibitors (TKI) as targeted therapy have shown remarkable success in advanced NSCLC (adenocarcinoma-ADK) patients with genetic alterations in epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK).

Detection of EGFR mutations and ALK rearrangements in ADK patients for TKI targeted therapy suitability.

DNA was extracted from FFPE primary/secondary lesions (284/116) obtained by biopsies/resections (120/280) from 400 unrelated Caucasian patients in different ADK stage (261M:139F) age between 28-89 years old, enrolled from February 2015-February 2016. Real-Time PCR method was performed for EGFR screening (exons 18-21). Subsequently, 15 EGFR-negative patients underwent FISH analysis for ALK/EML4 rearrangements detection. EGFR mutations were detected in 63 (15.75%) samples (39 primary/24 secondary tumors) mostly TTF-1 positive, tumor cell between 3%-90%. The TKI-sensitive mutations were mostly identified in exons 19 (53.98%) and 21 (L858R-28.57%, L861Q-4.76%) and less frequently in exon 18 (G719X-3.17%) and 20 (S768I-4.76%). The TKI-resistance mutations were detected in exon 20 as insertion type - primary resistance (3.17%) or as compound mutation del19/T790M - acquired resistance (1.59%). ALK/EML4 rearrangements were observed in 1 male patient with metastatic lesions (6.66%) who received anti-ALK targeted therapy.

Our results, in accordance with international data regarding the EGFR mutations and ALK rearrangements frequency, show a low frequency of the compound mutations.

The use of combined biomarkers like EGFR and ALK mutational profile may offer an accurate, efficient and cost-effective identification of lung ADK patients suitable for TKI-targeted therapy.

Acknowledgement: We thanks to Duzen Laboratories Group, Ankara, Turkey, for FISH analyzes.

## E-P12.072

**Association of miR-200b-3p and miR-1274a expressions in peripheral blood mononuclear cells with lung cancer**

C. Özbayır<sup>1</sup>, I. Değirmenci<sup>2</sup>, D. Üstüner<sup>3</sup>, G. Ak<sup>4</sup>, F. Saydam<sup>5</sup>, E. Çolak<sup>6</sup>, H. V. Güneş<sup>2</sup>, M. Metintas<sup>4</sup>;

<sup>1</sup>Dumlupınar University Kütahya School of Health, Department of Midwifery, Kütahya, Turkey, <sup>2</sup>Eskişehir Osmangazi University Faculty of Medicine, Department of Medical Biology, Eskişehir, Turkey, <sup>3</sup>Eskişehir Osmangazi University Vocational School Health Services, Department of Medical Laboratory, Eskişehir, Turkey, <sup>4</sup>Eskişehir Osmangazi University Faculty of Medicine, Department of Chest Diseases, Eskişehir, Turkey, <sup>5</sup>Recep Tayyip Erdoğan University Faculty of Medicine, Department of Medical Biology, Rize, Turkey, <sup>6</sup>Eskişehir Osmangazi University Faculty of Medicine, Department of Biostatistics, Eskişehir, Turkey.

Lung cancer with a poor prognosis and lower survival is a worldwide disease often accompanied by family history in around the world. The cases of lung cancer are often caused by long-term exposure to tobacco smoke, a combination of genetic factors and other forms of air pollution. Micro RNAs (miRNAs) are able to suppress the translation of mRNA into protein so have key roles in mechanisms of cancer cells. miRNAs are classified as oncogenic or tumor suppressor miRNAs depending on the functions of their mRNA targets in molecular pathways. It has reported that epigenetic mechanisms, such as DNA methylation and histone modifications, may be affected by this regulation. The miRNA group targeting the epigenetic control components called epi-miRNA. miR-200b-3p and miR-1274a are members of the epi-miRNA family. miR-200b-3p and miR-1274a target, respectively DNMT3a and DNMT3b. We aimed to determine the association of miR-200b-3p and miR-1274a gene expressions in peripheral blood mononuclear cells (PBMCs) with lung cancer. This study included 90 patients with lung cancer and 90 healthy controls. PBMCs were isolated by using Ficoll gradient centrifugation. Total RNA was extracted from these cells, and quantitative Reverse Transcriptase PCR (qRT-PCR) analysis of miR-200b-3p ve miR-1274a was performed as described in a commercial kit manufacturer's instructions. When miR-200b-3p and miR-1274a gene expression levels compared between the groups, both of them were decreased significantly in patients with lung cancer ( $P<0.05$ ). Our results suggest that miR200b-3p and miR1274a-1 may be tumor suppressors in lung cancer.

## E-P12.073

**Cytogenetics findings in patients with mature lymphoid neoplasm**

A. Strnkoval<sup>1</sup>, B. Drevojankova<sup>1</sup>, M. Stoklasova<sup>1</sup>, L. Truhlikova<sup>1</sup>, H. Kucharova<sup>1</sup>, A. Burdova<sup>1</sup>, T. Sablatureva<sup>1</sup>, M. Brejcha<sup>2</sup>, M. Wrobel<sup>2</sup>, D. Kłodova<sup>2</sup>, J. Duras<sup>3</sup>, D. Adamova<sup>4</sup>;

<sup>1</sup>Laboratoire AGEL a.s., Novy Jicin, Czech Republic, <sup>2</sup>Hospital Novy Jicin a.s., Novy Jicin, Czech Republic, <sup>3</sup>Faculty Hospital Ostrava, Ostrava, Czech Republic, <sup>4</sup>Hospital Opava, Opava, Czech Republic.

**Background:** Mature lymphoid neoplasms are an extremely heterogenous group of malignancies with regard to biological, clinical and morphological features. The identification of recurrent chromosomal changes has a major impact on the classification of mature lymphoid neoplasms.

**Patients and Methods:** We investigated 649 patients (354 men, 295 woman, average age 62) with primary mature lymphoid neoplasm in years 2010-2015 of which 68 were examined repeatedly. The conventional cytogenetic analysis (CCA) was performed on bone marrow samples using short time cultivation (24-48 hours) without stimulation. If it was required, interphase fluorescence in situ hybridization (I-FISH) was performed (most often to detect IGH, BCL2 or BCL6 rearrangement).

**Results:** We cultivated 98 % of samples successfully. Chromosomal aberrations were detected in 20 % of patients analyzed using CCA and I-FISH. CCA revealed chromosomal aberrations in 9 % of patients with negative I-FISH results.

**Conclusion/summary:** Our comparison with Overview of frequent and diagnostically relevant chromosomal aberrations in mature lymphoid neoplasms (Heim and Mitelman, 2015) will be discussed in the poster. According to our results it is important to combined both cytogenetic methods (CCA and I-FISH) to characterize recurrent chromosomal changes to correct classification of mature lymphoid neoplasms and suggest appropriate therapy.

## E-P12.074

**Association between Glutathione S-transferase P1 Ile105Val gene polymorphisms and Ann Arbor stage in Lymphoma**

G. Crauciuc<sup>1</sup>, F. Tripon<sup>1</sup>, A. Bogliş<sup>1</sup>, C. Duicu<sup>1,2</sup>, C. Bănescu<sup>1</sup>;

<sup>1</sup>University of Medicine and Pharmacy Tîrgu Mureş, Tîrgu Mureş, Romania, <sup>2</sup>1<sup>st</sup> Pediatrics Clinics, Clinical and Emergency Hospital, Tîrgu Mureş, Tîrgu Mureş, Romania.

**Introduction:** Glutathione S-transferase (GST) P1, T1, and M1 are phase II enzymes involved in metabolism of chemical carcinogens, drugs and xenobiotics. Because the etiological causes are not entirely known in lymphoma,

we sought a correlation between GST P1, T1 and M1 gene polymorphism according to risk of disease and evolution using Ann Arbor classification.

**Material and methods:** We enrolled 99 patients with different types of lymphoma and 200 control subjects without a malignancy history. Using PCR technique we determine the presence or absence of this gene.

**Results:** The average age for patients group is  $57.5 \pm 36.3$  and they represent all patients diagnose in the last 10 years in central region of Romania. The distribution of GST P1 Ile105Val genotype in patients group was: 70.7%Ile/Ile, 23.2%Ile/Val and 6.1%Val/Val and for GST T1/M1: 30.3%T1/M1, 38.3%T1/T1, 10%M1/M1 and 21.4%NULL. For control group GST P1 was found in the following percentage: 58.5%Ile/Ile, 33.5%Ile/Val and 8%Val/Val, and GST T1/M1 40.5%T1/M1, 41.5%T1/T1, 9.5%M1/M1 and 8.5%NULL. No difference was observed between investigated ( $p>0.05$ ). We noticed a positive correlation between GST T1/M1 gene polymorphisms and Ann Arbor staging in patients with lymphoma when we compare first stage with other three stage ( $p=0.022$ ; OR=1.235; 95%IC = 1.026-1.486).

**Conclusion:** These results suggest that GST T1/M1 and P1 polymorphism may not be considered a risk factor for occurrence of lymphoma, but GST T1/M1 may be involved in disease worsening and the absence of T1 or M1 allele can be correlated with a more advance stage of disease.

## E-P12.075

**BRAF V600E mutation in malignant melanoma**

P. Gurban<sup>1</sup>, F. Iordache<sup>1</sup>, S. Dinu<sup>1</sup>, R. Motoc<sup>1</sup>, G. Dinulescu<sup>1</sup>, A. Ungureanu<sup>2</sup>, P. Apostol<sup>1</sup>;

<sup>1</sup>Personal Genetics-Medical Genetics Center, Bucharest, Romania, <sup>2</sup>Radiotherapy Center Cluj, Cluj-Napoca, Romania.

A high proportion of human melanoma tumors harbor mutations in BRAF gene, V600E being the most frequent. Recent data confirm that BRAF V600E mutation is associated with significant clinical benefit of using BRAF-specific tyrosine kinase inhibitors.

The aim of our study was to estimate the frequency of BRAF V600E mutation in a study group of 245 patients with melanoma.

DNA was extracted from FFPE tissue samples and was amplified by PCR followed by reverse-hybridization. When detection performed by the above-mentioned method failed, samples were amplified using an in-house PCR followed by Sanger sequencing.

The mean age of the patients was 57 years old. Out of 245 patients (n =136 males and 109 females), 124 patients (50.61%) present the mutation in the BRAF gene. V600E mutation was found in 67 males (49.26 %) and 57 females (52.29%) respectively. From Fischer exact test (0.897) and of X2 square with Yates correction (0.222), no statistical significance ( $p<0.05$ ) between female patients and male patients with or without the mutation was found. The identification of mutant BRAF melanoma patients, who can benefit from BRAF-specific tyrosine kinase inhibitors treatment, is a step forward to personalized targeted therapy in metastatic melanoma which leads to improvement of the survival outcomes.

## E-P12.076

**The prevalence of the C677T polymorphism of methylenetetrahydrofolate reductase in acute lymphoblastic leukemia: A Tunisian experience**

R. Frikha;

Laboratory of Histology, Faculty of Medicine of Sfax, Sfax, Tunisia.

**Introduction:** Methylenetetrahydrofolate reductase (MTHFR) is the key enzyme for folate metabolism which play in DNA biosynthesis and the epigenetic process of DNA methylation. MTHFR gene polymorphism, mainly the C677T, have been implicated as risk factors for several types of cancers as the acute lymphoblastic leukemia (ALL). In addition, a potential impact of such variant in the efficacy of methotrexate has been reported.

In this study we evaluated the presence of the C677T variant of MTHFR in acute lymphoblastic leukemia patients from Tunisia. To provide new insights for a personalised therapy based on the human genotype.

**Materials and Methods:** Genomic DNA was extracted from EDTA-anticoagulant blood samples from a total of 35 patients suffering from ALL. Genotyping were carried out with restriction fragment length polymorphism (RFLP).

**Results:** The allelic frequency of the C677T variant of MTHFR was 13.6% in ALL patients, with a particular history of relapse and toxicity during methotrexate therapy.

**Conclusions:** Our findings suggest that C677T polymorphism of MTHFR is common in Tunisian ALL and affect clinical outcome. Thus, we insist that Genotyping of MTHFR polymorphism, C677T particularly, prior to treatment for ALL is likely to be useful in order to personalize therapy and reduce the MTX-related toxicities.

## E-P12.079

**Abnormalities of chromosome 1 in multiple myeloma**

M. Stoklasova<sup>1</sup>, L. Truhlikova<sup>1</sup>, A. Strnkova<sup>1</sup>, B. Drevojankova<sup>1</sup>, H. Kucharová<sup>1</sup>, A. Burdova<sup>1</sup>, T. Sablaturova<sup>1</sup>, C. Bodzاسova<sup>2</sup>, J. Duras<sup>2</sup>, J. Zuchnicka<sup>2</sup>, H. Plonkova<sup>2</sup>, I. Zygulova<sup>3</sup>, M. Brejcha<sup>4</sup>, M. Wrobel<sup>4</sup>;

<sup>1</sup>Laboratory of Medical Genetics – Department of Cytogenetics, AGEL Research and Training Institute – Novy Jicin Branch, AGEL Laboratories, Novy Jicin, Czech Republic,

<sup>2</sup>Institute of Clinical Hematology, University Hospital Ostrava, Ostrava, Czech Republic,

<sup>3</sup>Department of Hematology, Silesian Hospital in Opava, Opava, Czech Republic,

<sup>4</sup>Department of Hematology, Hospital Novy Jicin, Novy Jicin, Czech Republic.

**Background:** Multiple myeloma (MM) is a B-cell malignancy of the plasma cells characterized by complex cytogenetic aberrations. Chromosome 1 abnormalities are found in almost half of MM cases and are associated with aggressive disease. The most common structural changes that involve chromosome 1 are 1p deletion and 1q gain.

**Patients and Methods:** Between 2009-2014, 27 MM patients (6 women, 21 men, median age 68) showed chromosomal abnormalities by conventional chromosome banding (CCB). Bone marrow aspirates were processed using short time cultivation (24-48 hours) without stimulation. A minimum of IGH rearrangement and 13q deletion was performed in all cases by cytoplasmic immunoglobulin light chain staining (clg FISH).

Chromosome 1 abnormalities were confirmed by CCB, clg-FISH or FISH and multicolor FISH.

**Results:** Chromosome 1 aberrations were detected in 15/27 patients. Deletions were identified in 11/15 patients: 1p deletions as a result of unbalanced translocation (8/11), 1p interstitial or terminal deletions (2/11) and 1q deletion (1/11). Gains were caused by unbalanced translocation in 10/15 patients: 1q12-qter (5/10), 1q21-qter (3/10), 1q22-qter (1/10) and 1p21-qter (1/10). Trisomy 1 was presented in 1/15 patient. Deletion of 1p as well as gain of 1q were presented in 7/15 karyotypes.

**Summary:**

The 1p was preferentially involved in deletions that affect regions 1p11 and 1p13. The 1q gains were mostly in regions 1q12-qter and 1q21-qter. The 1q12-q23 region cover a large number of possible candidate genes. Our results support the importance of chromosome 1 abnormalities in the pathogenesis in MM.

## E-P12.080

**Evaluation of accompanying cytogenetic abnormalities and light chain profiles in 13q deleted MM patients**

C. D. Durmaz, T. Tuncali, N. Yürür Kutlay, Ö. Türedi, H. İlgin Ruhi, A. Vicdan, H. G.

Karabulut, A. Tükün;

Department of Medical Genetics, Faculty of Medicine, Ankara University, Ankara, Turkey.

Multiple myeloma (MM) is a hematological malignancy due to abnormal plasma cell infiltration in bone marrow. MM is the second most frequent hematologic neoplasm in Caucasians. The median age at onset is 66 years. Two-thirds of the patients have κ-light chain (κ-LC) type whereas the remaining one-third of the patients produce λ-light chain (λ-LC).

121 MM patients (61 men, 60 women, median age at onset 59 years) with del(13)(q) were evaluated for accompanied cytogenetic abnormalities and light chain profiles

In our study 38 patients (31,4%) had isolated del(13)(q) and the remaining 83 patients (68,6%) had additional cytogenetic abnormalities. The most frequent concomitant cytogenetic abnormality was IGH amplification (26,4%). Median survival (MS) was 4,1 years in isolated del(13)(q) group whereas was 2,8 years in the group with additional cytogenetic abnormalities. 60% of the patients' light chain was κ-LC and the MS was 3,6 years. However, the MS was 2,9 years among the λ-LC gammopathy patients (40%). In 34% of κ-LC gammopathy patients had isolated del(13)(q) and the MS was 4,3 years. The remaining patients (66%) had also another abnormalities and MS was 3,2 years. In 42% of λ-LC gammopathy patients had del(13)(q) alone and the MS was 3,8 years. The remaining patients (58%) had also another abnormalities and the MS was 2,2 years.

In our study we observed that MS was lower in λ-LC gammopathy and in the group with additional abnormalities accompanying del(13)(q).

Reference: Rajkumar and Kumar. „Multiple Myeloma: Diagnosis and Treatment.“ Mayo Clinic Proceedings. Vol.91. No.1. Elsevier, 2016.

## E-P12.082

**Ph - negative myeloproliferative neoplasm syndrome with acquired trisomy 21- case report**

O. Stanca<sup>1</sup>, M. Stoian<sup>2</sup>, C. Dobrea<sup>3</sup>, D. Barbu<sup>4</sup>, A. Ciobanu<sup>4</sup>, I. Triantafyllidis<sup>1</sup>, N. Berbec<sup>4</sup>, G. Petrica-Matei<sup>5</sup>, A. Lupu<sup>1</sup>;

<sup>1</sup>University of Medicine and Pharmacy „Carol Davila“, Coltea Clinical Hospital, Bucharest, Romania, <sup>2</sup>Personal Genetics-Medical Genetics Center, Discipline of Medical Genetics, University of Medicine and Pharmacy „Carol Davila“, Bucharest, Romania,

<sup>3</sup>OncoTeam Diagnostic, University of Medicine and Pharmacy „Carol Davila“ Bucharest-Fundeni, Bucharest, Romania, <sup>4</sup>Hematology Department - Coltea Clinical Hospital, Bucharest, Romania, <sup>5</sup>Personal Genetics-Medical Genetics Center, Bucharest, Romania.

The myelodisplastic/myeloproliferative neoplasms (MDS/MPNs) are a category of entities with intermediate features between myelodisplastic syndromes (MDS) and myeloproliferative neoplasms (MPNs), with clonal proliferation and chronic nature. The molecular pathogenesis of MDS/MPNs is complex and the diagnostic work up requires a full evaluation of clinical and morphologic findings, as well as cytogenetic and molecular analysis. Rare recurrent balanced abnormalities in Ph-negative MPNs involve PDGFRA, PDGFRB and FGFR1 rearrangements or other recurrent secondary abnormalities, such as trisomy 8 and trisomy 21. Acquired trisomy 21 is one of the most common numerical abnormalities in acute myeloblastic leukemia (AML), MDS and MDS/MPNs, but present as sole chromosomal abnormality in only 0,4% of cases.

We report a case of 59-year-old patient who presented with anemia, leukocytosis, splenomegaly. A bone marrow biopsy revealed a MDS/MPNs. An abnormal male karyotype (47,XY,+21[18]/46,XY[12]) with 60% mosaic trisomy 21, was revealed on GTG-banded metaphases obtained from bone marrow aspirate. Molecular testing using Real Time - PCR did not identify any BCR-ABL1 fusion transcripts. Fluorescent in situ hybridization (FISH) analysis using XL PDGFRA Probe (MetaSystems) revealed no arrangement. This case presents a challenge in terms of diagnosis, evolution and treatment options (cytoreductive therapy, tyrosine kinase inhibitors). Little is known about the pathogenic impact of the acquired trisomy 21 as a sole change in relation to disease type, morphologic subgroup, gender, and age. Using a multidisciplinary approach, with application of cytogenetic methods and molecular analysis plays an important role in the diagnosis, treatment and prognosis, allowing targeted therapy for the patients.

## E-P12.086

**Identification of the germline mutations of P53, CHEK2 and PTEN genes in non-BRCA1/2 mutation carrier high risk Turkish breast cancer families**

E. Mangoglu<sup>1</sup>, A. Moballegh<sup>1</sup>, M. Ozdogan<sup>2</sup>, C. Arici<sup>3</sup>;

<sup>1</sup>Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, Antalya, Turkey, <sup>2</sup>Antalya Medstar Hospital, Department of Medical Oncology, Antalya, Turkey, <sup>3</sup>Akdeniz University, Faculty of Medicine, Department of General Surgery, Antalya, Turkey.

Breast cancer is the most common malignancy in Turkey and worldwide. Approximately 5-10% of breast cancers are hereditary. In Turkish population BRCA1/2 mutations compose less than 10% of the hereditary breast cancer. For this reason studying the role of breast cancer susceptibility genes, in addition to BRCA1/2 is necessary. Among different breast cancer susceptibility genes P53, PTEN and CHEK2 are more stressed. In this study, 12 non-BRCA1/2 Turkish patients whose pedigrees are strong were chosen. Genomic DNA samples were isolated from peripheral blood. In P53 and PTEN genes, exons contain mutation hotspots that are exons 2, 3, and 5-8 in PTEN gene and exons 4-9 in P53 gene were amplified by PCR. In the case of CHEK2 gene whole coding exons (1-14) were amplified by PCR. All amplified exons were sequenced by capillary gel electrophoresis and screened for any possible mutation. As a result all of the 12 patients harbor the c.215C>G (p.Pro72Arg) polymorphism in heterozygous state on P53 gene. In 1 out of 12 patients c.638G>A (p.Arg213Gln) mutation in exon 6 of P53 gene was detected in heterozygous state. All patients were found to have heterozygous intronic c.802-4\_802-3delTT deletion in PTEN gene. Lastly, in one patient c.470T>C (p.I157T) mutation in the CHEK2 gene was detected in heterozygous state. In conclusion, in Turkish population these mutations may take part in breast cancer predisposition. But specifying their frequency and their amount of influence on breast cancer in Turkish population and on therapy need more studies including more cases and control group.

## E-P12.090

**The Effect of Propolis on the Cell Proliferation of Human Melanoma Cell Line**

N. Duran, G. Gulbul Duran, M. Urhan Kucuk; Mustafa Kemal University, Hatay, Turkey.

**Background and Aim:** Propolis is a bee product that contain hundreds of biologically active components in the structure. It has been used for centuries for treatment in folk medicine. It has numerous pharmacological properties such as; antimicrobial, antioxidative, anti-ulcerative and anti-tumor activities. We aimed to investigate the existence of antiproliferative effects of propolis on A375 (Human melanoma cell line) cells by epidermal growth factor (EGF) mRNA expression.

**Materials and Methods:** In study, vero cell line for cytotoxicity test as a control cells and Human melanoma cell line was selected (A375) for the study of anticancer activities were used. Non-cytotoxic concentrations of propolis were determined by the MTT method on Vero cells. Activity studies on A375 cells of propolis were investigated. The evaluation was performed as cell morphology, cell viability and mRNA expression levels. Methotrexate was selected as the standard drug (10 µg/mL).

**Results:** The cell viability and morphology of both Vero and A375 cells in various concentration were examined. Then non-cytotoxic concentration of propolis was determined up to 100 µg/mL on Vero cells. The growth of A375 cells was significantly suppressed in the presence of 25, 50 and 100 µg/mL of propolis. It was determined that propolis significantly reduce the mRNA expression levels at the concentration of 25, 50 and 100 µg/ml when it is compared with the control group ( $p < 0.001$ ).

#### Conclusion

In the study, propolis inhibit the proliferation of A375 cells in relation to dose-time and likewise EGF mRNA levels were significantly reduce. We think that propolis may be contain available active components in cancer therapy.

#### E-P12.091

#### Investigation of Autophagy Gene ATG16L1 Polymorphism in Human Prostate Cancer and Bladder Cancer

*S. Budak Diler<sup>1</sup>, F. Aybuğ<sup>2</sup>:*

<sup>1</sup>University of Niğde, Faculty of Science and Letters, Department of Biotechnology, Niğde, Turkey, <sup>2</sup>University of Niğde, Natural and Applied Sciences Institute, Department of Biology, Niğde, Turkey.

**Introduction:** Urological cancers (prostate cancer and bladder cancers) are the most common cancers in Western population and its rate is increasing in the Eastern World. Autophagy has appeared as a fundamental repair mechanism for degrading damaged organelles and proteins. It was clear that autophagy gene polymorphisms are correlated with development of inflammatory bowel disease and it can also be related with prostate cancer (PCa) or bladder cancer (BCa).

**Aim:** In this study, we aimed to determine if ATG16L1 (Thr300Ala) polymorphism is associated with an increased risk of developing PCa and BCa and to establish correlations between ATG16L1 genotypes and morphological parameters.

**Method:** This study included 269 healthy controls and 131 patients (62 PCa and 69 BCa) with PCa and BCa. The ATG16L1 (rs2241880) gene regions were amplified using polymerase chain reaction (PCR), detected by restriction fragment length polymorphism (RFLP).

**Results:** At the end we found out that the genotype AG was prevalent on patients and controls (34% vs 42%), followed by genotypes AA (35% vs 27%) and GG (31% vs 31%) in PCa. The prevalence of genotypes of AA (wild-type), AG (heterozygous mutant) and GG (homozygous mutant) profiles for the Atg16L1 Thr300Ala polymorphism were 35%, 40% and 25% respectively in BCa patients, and 32%, 40% and 28% respectively in healthy control groups. The G allele frequency was 0.53 for in BCa patients and the control groups.

**Conclusion:** Any association was not found for ATG16L1 (Thr300Ala) polymorphism between patients with PCa and BCa and control groups in Turkish population.

#### E-P12.092

#### Complex genetic testing of colorectal cancer

*K. Zavodna<sup>1</sup>, J. Markus<sup>1</sup>, A. Alemanyehu<sup>1</sup>, T. Slamka<sup>1</sup>, R. Lohajova Behulova<sup>2</sup>, O. Hamidova<sup>2</sup>, M. Konecny<sup>2</sup>:*

<sup>1</sup>St. Elizabeth Cancer Institute, Bratislava, Slovakia, <sup>2</sup>Institute of Genetics and Molecular Medicine, Faculty of medicine, Slovak Medical University in Bratislava, Bratislava, Slovakia.

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, representing the second most frequently occurring cancer among females and the third among males.

The presence of the RAS mutations in colorectal cancer patients correlates with lack of response to the certain EGFR inhibitor therapies. We used the SNaPshot analysis to detect somatic mutations in KRAS/NRAS (codons: 12, 13, 59, 61, 117, 146) and BRAF genes (codon 600).

The microsatellite instability (MSI) phenotype represents ~15% of all CRCs and is caused by deficient DNA mismatch repair mechanism (MMR), which is a consequence of germline mutations in MMR genes or epigenetic silencing of the MLH1 gene. We used the MSI analysis for universal colorectal tumor testing: 1. MSI-H status is a marker of favourable prognosis and not benefit from 5-fluorouracil-based adjuvant chemotherapy in stage II. 2. MSI-H status is the hallmark of HNPCC-associated tumors.

Approximately 5-10% of all CRC have hereditary background and the most common is Lynch syndrome (LS). Patients with LS are carriers of a mutation in MMR genes. Identification of a mutation is not only important for a patient, but also for his or hers relatives. We used the standard Sanger sequencing analysis to detect germline mutations in MMR genes. Currently we are using the next generation sequencing approach, we are able to test a panel of 26 genes (Illumina MiSeq).

Our complex approach to genetic testing of patients with colorectal cancer allows us to differentiate patients and provide them with personalized clinical management.

#### E-P12.093

#### Two congenital reciprocal translocations in family with hematologic diseases

*S. Ransdorfova<sup>1</sup>, J. Brezinova<sup>1</sup>, Z. Zemanova<sup>2</sup>, I. Sarova<sup>1</sup>, H. Lhotska<sup>2</sup>, M. Lauermanova<sup>1</sup>, A. Baxová<sup>3</sup>, M. Valerianova<sup>3</sup>, K. Michalova<sup>1,2</sup>:*

<sup>1</sup>Institute of Hematology and Transfusion, Prague 2, Czech Republic, <sup>2</sup>Center of Oncocytogenetics, Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and 1st Faculty of Medicine, Charles University, Prague 2, Czech Republic, <sup>3</sup>Institute of Biology and Medical Genetics, General University Hospital and 1st Faculty of Medicine, Charles University, Prague 2, Czech Republic.

Acquired balanced chromosomal aberrations are associated with many types of hematologic malignancies leading to critical gene fusions or other gene alterations. However, the role of constitutional chromosomal abnormalities in hematologic malignancies has not been as yet clarified. Here we present an unusual case of two congenital balanced translocations found in members of a family with hematologic diseases.

We examined bone marrow of 58 years old woman with chronic lymphocytic leukemia with conventional and molecular cytogenetic techniques (FISH, mFISH/mBAND, aCGH/SNP). Chromosome analysis showed an abnormal karyotype 46,XX,t(2;12)(q24;q21),der(5)t(5;14)(q12;q31),der(14)inv(q21q31)t(5;14)(q12;q31) in all cells analyzed. Congenital origin of rearrangements was confirmed in peripheral blood lymphocytes. The same karyotype was demonstrated also in a 24-year-old daughter with essential thrombocythemia. Moreover, we examined constitutional karyotype of other two daughters. In 32-year-old woman we found a sole der(5)t(5;14)(q12;q31),der(14)inv(q21q31)t(5;14)(q12;q31), whereas in the 18-year-old one we proved a sole translocation t(2;12)(q24;q21). The involved genes have not been described so far in association with hematologic diseases. All four women have normal phenotype, however they are treated for endogenous depression.

The occasional observation of constitutional chromosomal abnormalities in patients with malignant disease raises a question of their potential role in cancer development. It is unclear whether the constitutional aberrations, especially reciprocal translocation, are a coincidental feature or a predisposing factor in the development of the malignant disease. Therefore, the documentation of more patients with constitutional structural aberrations and hematologic diseases could help to the identification of new specific genes/regions potentially implicated in tumorigenesis.

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#### E-P12.096

#### Association of common variants in luteinizing hormone gene and its receptor with testicular cancer: a Czech pilot study

*J. Chrudimská, A. Brisuda, M. Macek jr, M. Macek sr.:*

Charles University - 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic.

**Introduction:** There are two common variants in LH $\beta$  subunit gene Trp8Arg (rs1800447) and Ile15Thr (rs34349826) that form together variant  $\beta$  subunit of LH protein (v-LH $\beta$ ) with higher bioactivity (tests in vitro), but shorter half-life in vivo. In LHCGR gene, there are common polymorphisms with an impact on receptor function - ins18LQ (rs2293275), p.Asn291Ser (rs12470652) and p.Ser312Asn (rs2293275). Testicular cancer (TC) primarily occurs in younger men with hormonal disturbances in testes. Certain polymorphisms in LHCGR and ESR1 genes were associated with a higher risk of TC. The aim of this study was to ascertain the association of selected variants in LH $\beta$  and LHCGR genes with the risk of TC.

**Materials and Methods:** DNA from 35 Czech patients with TC were genotyped using RFLP for detecting v-LH $\beta$ , fragment analysis for ins18LQ and TaqMan assays for p.Asn291Ser and p.Ser312Asn variants. Results were compared to 101 unrelated Czech fertile male controls without TC.

**Results:** The v-LH $\beta$  variant protects from developing TC:  $P=0,08187/0,01901$  (for genotype/allelic frequencies, respectively), odds ratio 0,27189 (95% CI 0,06116758-0,8030376). No difference in genotype/allelic

frequency in TC patients and in control men in LHCGR variants: P=0,3795 / P=0,5244 for insLQ; P=0,1583 / P=0,1689 for Asn291Ser and P=0,6284 / P=0,7822 for Ser312Asn (P values for genotype/allelic frequencies, respectively).

Conclusions: Although our initial results should be confirmed on a larger sample, presence of v-LH $\beta$  allele protects from developing of TC, while no association with variants in LHCGR gene was found.

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#### E-P12.097

#### Thr241Met Polymorphism of XRCC3 Gene is not Associated with Lung Cancer Risk in Romanian Population

A. Catana<sup>1</sup>, B. Hancu Domokos<sup>2</sup>, I. Blaga<sup>1</sup>, D. Margineanu<sup>1</sup>, Z. Cuzmici<sup>1</sup>, F. Petrisor<sup>1</sup>, R. Pop<sup>1</sup>;

<sup>1</sup>Department of Medical Genetics, University of Medicine and Pharmacy, Cluj Napoca, Romania, Cluj Napoca, Romania, <sup>2</sup>Department of Pneumology, University of Medicine and Pharmacy, Cluj Napoca, Romania, Cluj Napoca, Romania.

**Background and Aims:** DNA repair mechanisms play a critical role in protecting the cellular genome against carcinogens. X-ray cross-complementing gene 3 (XRCC3), is involved in DNA repair and therefore, certain genetic polymorphisms that occur in DNA repair genes may affect the ability to repair DNA defects and may represent a risk factor in carcinogenesis. The purpose of our study was to investigate the association between XRCC3 gene Thr-241Met polymorphism and the risk of lung cancer, in a Romanian population.

**Methods:** We recruited 93 healthy controls and 85 patients with lung cancer, all smokers Thr241Met, XRCC3 gene genotyping was determined by multiplex PCR-RFLP.

**Results:** Statistical analysis (OR, recessive model), did not revealed an increased risk for lung cancer, for the variant 241Met allele and Thr241Met genotypes (p=0.138, OR=0.634, CI=0.348-1.157; p=0.023, OR=0.257, CI=0.085-6.824). Also, there were no positive statistical associations between Thr241Met polymorphism of XRCC3 gene, gender and various histopathological tumor type of lung cancer.

**Conclusion:** In conclusion, the results of the study suggest that the XRCC3 gene Thr241Met polymorphism is not associated with an increased risk for the development of lung cancer in Romanian patients.

#### E-P12.098

#### miRNA Expression Testing of Thyroid Fine-Needle Aspirations Could Improve Presurgical Diagnosis

D. Usaite<sup>1,2</sup>, K. Suziedelis<sup>1,2</sup>, L. Kunigenas<sup>1,2</sup>;

<sup>1</sup>National Cancer Institute, Vilnius, Lithuania, <sup>2</sup>Joint Life Science Center, Vilnius University, Vilnius, Lithuania.

**Introduction:** Discrimination between malignant follicular thyroid cancer (FTC) and benign follicular thyroid adenoma (FTA) is the most difficult aspect of thyroid pathology. Fine needle aspiration cytology (FNAC) has emerged as a successful diagnostic test and has reduced the number of unnecessary thyroidectomies. However, only 30% of patients who are reported as Follicular neoplasm/Suspicious for follicular neoplasm or Suspicious for malignancy by cytopathology and have thyroidectomy or surgical lobectomy, reported as Follicular carcinoma by postsurgical histopathology.

**Material and methods:** 23 patients' miRNAs were extracted from fine needle aspirations and FFPE biopsies using "miRNeasy Serum/Plasma kit" and "miRNeasy FFPE Kit" accordingly. FFPE and FNA samples were distributed into FTC and FTA. miRNA expression levels in follicular thyroid tumors and adenomas were performed by RT-PCR using miRNome miRNA Custom Assay PCR Array. All data were analysed and compared using qPCR data analysis software.

**Results:** 13 miRNAs whose expression increased or decreased more than 1,5-fold in FFPE FTC compared to FFPE FTA were identified. All these selected miRNAs were run and compared in malignant and benign fine needle aspiration samples. The results showed the expression of 3 miRNAs were up- or down- regulated more than 1,5-fold in malignant FNAs.

**Discussion:** At present accurate presurgical thyroid follicular nodules' diagnosis is not satisfactory. We have found that 3 miRNAs were differentially expressed in FTC compared to benign nodules. Two up-regulated (miR-30a, miR-122) and one down-regulated (miR-93). These data suggest that FTA can be distinguished from FTC by miRNA expression analysis.

#### E-P12.099

#### Pharmacogenetic variants in patients with thyroid cancer

Z. A. Hammoudeh<sup>1</sup>, D. Nikolova<sup>1</sup>, L. Balabanski<sup>2</sup>, S. Ivanov<sup>2</sup>, R. Vazharova<sup>2,3</sup>, S. Weidner<sup>4</sup>, M. Malinov<sup>2</sup>, D. Toncheva<sup>1,2</sup>;

<sup>1</sup>Department of Medical Genetics, Medical University of Sofia - Bulgaria, Sofia, Bulgaria,

<sup>2</sup>SBALGAR "Malinov", Sofia, Bulgaria, <sup>3</sup>Medical Faculty, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria, <sup>4</sup>Department of Nuclear Medicine Inselspital, Berne University Hospital, Berne, Switzerland.

**Introduction:** Thyroid cancer is the most common malignancy of endocrine organs. Kinase inhibitors may be used as target therapy of thyroid cancer with mutations in EGFR and RET genes. The effect of pharmacogenetic variants on the chemotherapy of papillary thyroid cancer are not widely studied. **Methods:** We performed DNA analysis on 10 patients with papillary thyroid cancer. All patients provided a signed written informed consent for examination of their samples. Genomic DNA was extracted from blood samples and was sequenced by NGS instrument (Illumina-MiSeq) using the TruSight cancer panel of 94 genes and 284 SNPs. The results were analyzed using pharmgkb database (<https://www.pharmgkb.org/>) for SNP variants associated with sensitivity to certain drugs.

**Results:** Pharmacogenetic variants were found in different genes: ERCC2 (rs13181), ERCC5 (rs17655), TP53 (rs1042522) and XPC (rs2228001). These variants are sensitive to chemotherapy with the platinum compounds (cisplatin). Additional important variants in EGFR (rs227983) and RET (rs1799939) genes were found, which define thyroid cancer as sensitive to target therapy with tyrosine kinase inhibitors such as gefitinib and sunitinib.

**Conclusion:** Our data shows that cancer panel has future clinical application and could lead to further personalization of treatment. It has the potential to improve the therapy outcome and prognosis for thyroid cancer.

#### E-P12.101

#### MOTIEF study: identification of germline MutatiOns linked To urinary bladder cancer In Early-onset patients and Families

A. J. Grotenhuis, S. H. Vermeulen, T. E. Galesloot, R. P. Kuiper, L. A. L. M. Kiemeney; Radboud university medical center, Nijmegen, Netherlands.

**Introduction:** A positive family history of urinary bladder cancer (UBC) doubles the risk for UBC and suggests a role for germline genetic variants in disease aetiology. Common UBC susceptibility variants were already successfully identified through genome-wide association studies, but little is known about rare genetic variants underlying UBC predisposition. The objective of MOTIEF is to identify high-penetrance germline mutations predisposing for UBC by using a unique series of early-onset patient-parent trios and striking families.

**Materials and Methods:** This study consists of three parts: 1) recruitment of extremely early-onset ( $\leq 30$  years) sporadic UBC patients diagnosed since 1989 and their non-affected parents (trios) based on Netherlands Cancer Registry data, and biomaterial collection (blood/tumor) of these trios and 64 known high-risk UBC families; 2) identification of novel candidate UBC-predisposing mutations and genes through whole-genome sequencing (WGS) of germline DNA of stringently selected affected relatives from high-risk families (N~145) and the trios (N~150); 3) substantiation of novel candidate UBC-predisposing genes through high-throughput mutational screening for an anticipated 100 WGS-selected candidate genes using molecular inversion probe (MIP) technology. Also, we will screen collected tumor DNA for second-hit mutations and somatic changes in the selected genes. Finally, validated UBC-predisposing genes will be studied in relation to UBC subtypes.

**Expected results:** a) The identification of rare, novel, high-penetrance germline mutations predisposing for familial and early-onset UBC and second-hit (somatic) mutations, b) their influence on UBC subtypes, and c) the establishment of an early-onset and familial UBC biobank for future research.

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#### E-P12.104

#### A novel frameshift mutation in Bulgarian patient with von Hippel-Lindau syndrome

M. Glushkova<sup>1,2</sup>, I. Yordanova<sup>1,2</sup>, T. Todorov<sup>2</sup>, A. Todorova<sup>1,2</sup>, V. Mitev<sup>1</sup>;

<sup>1</sup>Department of Medical Chemistry and Biochemistry, Medical University Sofia, Sofia, Bulgaria, <sup>2</sup>Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria.

Von Hippel-Lindau syndrome (VHL) is an autosomal dominant disease with incidence estimated as 1:36,000 live births. The mean age of diagnosis is 28 years. It is characterized by hemangioblastomas in the central nervous system and/or retina, renal cell carcinomas, phaeochromocytomas, pancreatic tumors, endolymphatic sac tumors, epididymal and broad ligament

cystadenomas. In 80% of the cases hemangioblastomas are developed in the brain, while in the rest 20% - in the spinal cord and in some rare cases in the peripheral nerves.

Here we report seven unrelated Bulgarian patients diagnosed as VHL. The genetic testing included direct sequencing and MLPA. In four patients out of 7 (57%) the molecular defect was detected: one small deletion, one point mutation, one nonsense mutation and one novel frameshift mutation, which is indel.

The patient with a novel frameshift indel is 27 years old woman with clinical characteristics of hydrocephalus, perifocal edema of the brainstem and cerebellar vermis. The doctors suggested that the tumor formation in fourth ventricle is probable ependymoma or hemangiopericytoma. Tumor formations in other part of patient's body are absent. The detected novel molecular defect in exon 3 of the VHL gene is a complex indel c.[516\_517insAGTCAAGCCT; 532\_542delCTGGACATCGTinsATTA], p.Glu173Serfs\*4. The possible mechanism for mutation generation involves the genetic architecture of the neighboring area rich in repeated elements.

The genetic testing provides the possibility for adequate genetic counseling and prenatal diagnostics in affected families.

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## E-P12.105

### XPF -673C>T and XPF 11985A>G gene polymorphisms and risk of chronic myeloid leukemia

C. Banescu, V. G. Moldovan, F. Tripone, A. G. Crauciuc, C. Duicu, A. Boglis, M. Doboreanu; University of Medicine and Pharmacy Tg. Mures, Romania, Tg. Mures, Romania.

Xeroderma pigmentosum group F (XPF) genes are implicated in nucleotide excision repair (NER) system, one of the most important DNA repair system. Polymorphisms of this genes may influence the capacity of DNA repair system and therefore may predispose to malignancy, including leukemia. We performed a case-control study to investigate the relation between XPF -673C>T, XPF 11985A>G and chronic myeloid leukemia (CML) in a Romanian population. We investigated 82 adult CML patients which were Ph1 / BCR-ABL positive and 170 subjects with no history of cancer. We found no association between several prognostic factors such as blasts, basophils, additional chromosomal abnormalities and XPF -673C>T, XPF 11985A>G polymorphisms in our CML cohort. No differences were found when we examined the distribution of variant genotypes of XPF gene polymorphisms according to EUTOS score. We also looked whether there was any relation between investigated XPG polymorphisms and treatment, but no association was noticed. According to our data XPF -673C>T and XPF 11985A>G do not represent risk factors for development of CML. We consider that further study on a larger CML cohort is required to validate our observations.

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## E-P13 Basic mechanisms in molecular and cytogenetics

### E-P13.01

#### De Nova 18p Deletion Syndrome that Diagnosed at Seven Years Old Boy

S. Canbek<sup>1</sup>, A. Ayaz<sup>1</sup>, T. Celik<sup>2</sup>;

<sup>1</sup>Adana Numune Training and Research Hospital, Clinical Genetics Department, Adana, Turkey, <sup>2</sup>Adana Numune Training and Research Hospital, Pediatric Neurology Department, Adana, Turkey.

Introduction: The deletion 18p syndrome is one of the most common chromosome abnormalities including variously phenotypic properties. Mental retardation, failure to thrive, round face, dysplastic ears, wide mouth and anomaly of teeth, eye, brain, heart and genital system are mostly seen clinical features. We herein report seven years old boy with dysmorphic face features, mild mental retardation, dental developmental deficiency.

Materials and Methods: Chromosome analyses and array comparative genomic hybridization (array-CGH) analyses have done. Results: Patient has diagnosed as 18p deletion syndrome by peripheric chromosome analysis. The karyotypes of the child and her parents were subjected to G-banding chromosome analysis, and array comparative genomic hybridization (array-CGH) was used for fine mapping of the aberrant region. His parental chromosome analysis were normal. Array CGH has identified a 18,4 Mb deletion at 18p11.22 region.

Conclusion: This case reported for genotype-phenotype correlations.

### E-P13.02

#### A Case: mild clinical signs with tetrasomy 9P

B. Eroglu Kesim<sup>1</sup>, K. Yararbas<sup>2,3</sup>, S. Kasakyan<sup>2</sup>, K. Yüksek Acinikli<sup>4</sup>;

<sup>1</sup>Sisli Etfal Research Hospital, İstanbul, Turkey, <sup>2</sup>Duzen Laboratories Groups, İstanbul, Turkey, <sup>3</sup>Maltepe University, İstanbul, Turkey, <sup>4</sup>Bahcelievler Goverment Hospital, İstanbul, Turkey.

Tetrasomy 9p, or supernumerary isochromosome 9p [i(9p)], is a rare chromosome abnormality resulting from a supernumerary isochromosome 9p. The tetrasomy 9p phenotype varies in severity from neonatal death to mild developmental delay and minor anomalies. Abnormal genitalia were also complicated in 56% of male case, and 38% of female case. The phenotypic variability of T9p is largely unexplained. It has been suggested that the severity of the phenotype might be related to the degree of mosaicism. The influence of breakpoints position on the severity of phenotype is controversial. Our The patient, a 6 year-old girl, was child of consanguineous healthy parents. Delivery was at 41 weeks of gestation with a birth weight 3150 g, birth length 50cm, and head circumference 33cm. She had imperforate hymen. She has prominent forehead, short philtrum, downslanting corners of the mouth, micrognathia, low-set ears, incomplet cleft lip, abnormal enamel and Cardiac ECHO showed slightly mitral failure.

The patient's karyotype was 47,XX,+der 9 [90]/ 46,XX, [10]. FISH studies of lymphocytes were performed to confirm the origin of this isochromosome, using the whole chromosome 9-specific painting probe (WCP9) and the result was compatible with isochromosome 9p. We performed molecular karyotyping and patient's result was 47, XX, idic (9) (q13), arr[hg19]9p24.3q13(612,166-68,283,133)x4.

Our case has mild clinical signs, despite the high level of mosaicism and 9q13 breakpoint.

### E-P13.03

#### Antagonistic effect of oxytocin and tacrolimus combination on adipose tissue-derived mesenchymal stem cells

B. Goker<sup>1</sup>, G. Sir<sup>2</sup>, G. Yigiturk<sup>3</sup>, T. Cavusoglu<sup>3,4</sup>, C. Biray Avcı<sup>1</sup>, C. Gunduz<sup>1</sup>, Y. Uyanikgil<sup>2,3,4</sup>;

<sup>1</sup>Department of Medical Biology, School of Medicine, Ege University, Bornova, Izmir, Turkey, <sup>2</sup>Department of Stem Cell, Institute of Health Science, Ege University, Bornova, Izmir, Turkey, <sup>3</sup>Department of Histology and Embryology, Faculty of Medicine, Ege University, Bornova, Izmir, Turkey, <sup>4</sup>Cord blood, Cell-Tissue Research and Application Center, Ege University, Bornova, Izmir, Turkey.

Introduction: In this study we aimed to eliminate the negative effects of tacrolimus (immunosuppressive drug) on adipose tissue-derived mesenchymal stem cells (AT-MSC) by using an antioxidant agent oxytocin.

Material-Method: AT-MSC cell line was obtained commercially and 5<sup>th</sup> passage of the original line was obtained. To evaluate the cytotoxic effects of tacrolimus and oxytocin on AT-MSC, WST-1 test was applied. Also Isobogram analyses were conducted to evaluate combinational effects of tacrolimus and oxytocin. After calculating the IC<sub>50</sub> values of tacrolimus and oxytocin and combination indexes, determined doses were given to cells and apoptosis analyses were conducted in 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> period. Study groups were tested using Muse Cell Analyzer with Muse<sup>®</sup> Annexin V and Dead Cell Assay Kit for determining viability, apoptosis and death cell numbers. Immunofluorescence staining of Ki67 proliferation and vimentin stem cell marker in untreated AT-MSC cell line. AT-MSCs treated with IC<sub>50</sub> values of tacrolimus, oxytocin and the combination of both for 96 hours. Nuclear staining was visualized using DAPI.

Results: IC<sub>50</sub> values for oxytocin and tacrolimus were 17.44 $\mu$ M and 13.43 $\mu$ M, respectively. Combination indexes of Isobogram analysis revealed that the two agents were antagonistic. Ki67 immunoexpression of tacrolimus group significantly lower compared with negative control group but tacrolimus-oxytocin combination group similar to negative control group. Ki67 immunoexpression of oxytocin group significantly higher than the other groups. Vimentin immunoexpression of oxytocin group significantly lower than the other groups.

Conclusion: The results clearly points out that, oxytocin could be used to eliminate the cytotoxicity caused by tacrolimus.

### E-P13.04

#### Investigation of the PTPN22 gene polymorphism in alopecia areata

S. D. Celik<sup>1</sup>, O. Ates<sup>1</sup>, N. Bozkurt<sup>1</sup>, S. Sezer<sup>1</sup>, E. Ensari<sup>1</sup>, G. Kalkan<sup>2</sup>;

<sup>1</sup>Department of Medical Biology, Medical Faculty, Gaziosmanpasa University, Tokat, Turkey, TOKAT, Turkey, <sup>2</sup>Department of Dermatology, Medical Faculty, Yildirim Beyazit University, Ankara, Turkey, TOKAT, Turkey.

Introduction: Alopecia areata (AA) is a common, chronic, inflammatory disease that characterized by various size and shapes of hair loss lesions on the scalp and/or body. However the cause of AA is not entirely understood in present, it is considered to be a T-lymphocyte mediated autoimmune disease. The protein tyrosine phosphatase, non-receptor type 22 (PTPN22) have important roles in the physiology of hair follicle is a gene. The PTPN22

gene encodes a lymphoid protein tyrosine phosphatase (LYP) which acts as a negative regulator of T cell signaling. The impaired function of PTPN22 in T cells may induce the production of autoantibodies and leading to development of autoimmune diseases. The aim of our study was to investigate the effect of PTPN22 C1858T polymorphism on the predisposition to AA.

**Materials and Methods:** The present study analyzed the genotype distribution and allele frequency for the C1858T polymorphism using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique in 190 AA patients and 161 healthy individuals.

**Results:** The genotype and allele distribution of PTPN22 C1858T polymorphism have not been associated with AA ( $p=0.055$  and  $p=0.057$ , respectively). The homozygous TT genotype was not identified in the AA patients and control groups.

**Conclusions:** The results do not support the hypothesis that the C1858T (R620W) polymorphism of the PTPN22 gene is an important risk factor for AA. This study was supported by Gaziosmanpaşa University Scientific Research Projects Fund.

#### E-P13.05

#### Rare chromosomal aberrations in Iranian patients with Aplastic Anemia

B. Sadeghi<sup>1,2</sup>, V. Yassaee<sup>1,3</sup>, Z. Ravesi<sup>1</sup>, M. Aghighi Rad<sup>1</sup>, M. Ekrami<sup>1</sup>, P. Gholami<sup>1</sup>;

<sup>1</sup>Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of; <sup>2</sup>shahid beheshti university, Tehran, Iran, Islamic Republic of; <sup>3</sup>Dept. of Medical Genetic, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Aplastic anemia is a rare blood disease with poor prognosis. It is difficult to distinguish from myelodysplastic syndrome using conventional laboratory blood tests. Bone marrow cytogenetic studies may have an advantage for discriminating the former from the latter one.

During the past five years, we examined bone marrow and blood specimens derived from over 300 blood cancer patients. Here, we report three cases with rare chromosomal aberrations.

A 27 years old female presented with thrombocytopenia, pancytopenia and her chromosomal analysis showed 46 XX and 47, XX, + 21 for blood and bone marrow respectively.

A 25 years old female manifested initial diagnosis of anaplastic lymphoma and pancytopenia. Cytogenetic study revealed 46 XX for blood and 46,XX,t(1;2)(p21.1;p12)[18]\46,XX,t(2;8;11)(p22;p23.2;q24.2)[14]\46,XX,t(4;8)(p32;q24.3)[4]/46,XX,t(13;18)(q11.2~q13; p11.3)[8] for bone marrow.

A 30 years old man with pancytopenia was clinically diagnosed for acute leukemia. Chromosomal analysis revealed 46 XX for blood and 46 XY, t(6;9)(p23; q33) in bone marrow.

None of the above cases showed any pattern of chromosome abnormality as observed in Fanconi Anemia. In this case karyotyping is beneficial for considering the best possible treatment. This approach substantially ignores the necessity of bone marrow transplantation.

Our results accentuate the advantages of cytogenetic studies of bone marrow specimens over peripheral blood of leukemia patients. An efficient diagnostic plan is paramount because time from diagnosis to treatment is critically related to outcome regardless of the elected therapeutic option.

#### E-P13.06

#### Chromosomal Microarray Analysis (CMA) and Fluorescence In-Situ Hybridization (FISH) revealed a rare case of de novo deleted and duplicated segments of 6q25.1->q26 in an infant presented with motor delay, dysmorphic features and enlarged cisterna magna

M. L. Tan, H. J. Ee, R. S. Roch, H. Y. Yon, S. A. C. Ling, L. T. Toh, M. S. Brett, H. Y. Law, S. S. Jamuar, M. H. Yong, A. H. M. Lai;

KK Women's And Children's Hospital, Singapore, Singapore.

**Introduction:** Interstitial deletions with duplications of the long arm of chromosome 6 are rare. We report an infant with motor delay, dysmorphic features, hypotonia and enlarged cisterna magna with both deletion and duplication segments in 6q25->q26.

**Materials and Methods:** CMA was performed on the proband using Agilent Technologies 4x180K SurePrint G3 Human CGH+SNP Platform and Cyto-genomics 2.5 software. High resolution G-banding was carried out on stimulated lymphocytes cultures. Targeted FISH was done using RP11-43B19, RP11-1112G6 and RP11-455I14 BAC probes. Parental FISH and chromosome analysis were also performed.

**Results:** CMA on the proband revealed a pathogenic loss of 3.11Mb at 6q25.3 (hg19:157,261,472-160,368,328). This deletion affects 16 OMIM including ARID1B, SERE1, GTF1H5, SOD2 and ACAT2. CMA also showed a

loss of 0.48Mb at 6q25.3q26 (hg19:160,800,432-161,284,434) and a gain of 3.75Mb at 6q25.1q25.3 (hg19:152,046,289-155,794,132), which are of uncertain clinical significance. Chromosome analysis showed a female karyotype with a derivative chromosome 6 resulting from a deletion of 6q25.3->q26 and a duplication of 6q25.1->q25.3. Metaphase FISH with RP11-43B19 and RP11-1112G6 showed deleted signals while RP11-455I14 showed duplicated signals on the same derivative 6. No apparent abnormality was found in both parents.

**Conclusions:** Interstitial deletions in 6q25 have been associated with microcephaly, developmental delay, dysmorphic features, hearing loss and agenesis of the corpus callosum. However, the clinical significance of the 0.48Mb loss and 3.74Mb gain are unclear. This case demonstrated the usefulness of FISH and karyotyping in elucidating the complex chromosomal rearrangement. FISH is also a cost-effective way to determine if the abnormality is inherited.

#### E-P13.07

#### Complex chromosomal rearrangement in a patient with periconceptional exposure to ionizing radiation

M. A. Orera, C. Rodriguez Hernandez, B. Marquez Arce, M. Moreno Domene, M. Prieto, R. Herranz Crespo;

Hospital Gregorio Marañón, Madrid, Spain.

**Introduction:** Ionizing radiation (IR) generates cellular damage through the interaction with DNA where the energy transfer induces structural breaks. The nonhomologous DNA end joining repair can originate chromosomal stable translocations. Periconceptional exposure to high dose of IR usually results in abortion, and lower doses, could be related to development of congenital malformations.

**Materials and Methods:** We present a 28 years old patient, born in Romania in 1986, 800 Km from Chernobyl. She has hypernasal speech and mild dyspraxia. She has had three spontaneous abortions before the 6<sup>th</sup> gestational week. Parents are non related. Her father has been diagnosed of hairy cell leukaemia, BRAF positive at 53 years of age.

**Results:** The peripheral blood karyotype revealed a complex chromosome rearrangement that required six double strand breaks: 46,XX, inv(1)(q23;q32); del(2)(q33-q35);der(18)der(14),t(2;18;14)(18pter-q21.3::2q32-q35::14q23-pter) (14pter-q23::18q21-pter). Array CGH at 60K resolution was normal, showing no gain or losses. The mother has an inversion in the long arm of chromosome 1, and her father's karyotype is normal. FISH based genomic translocations frequency adjusted for her individual characteristics, once the constitutional translocations are excluded, is normal.

**Conclusions:** The patient was conceived in the aftermath of the Chernobyl nuclear accident. Her peripheral blood shows chromosomal rearrangement, which points to a single event occurring early in the development. The probability of the appearance of the rearrangement due to three independent events is  $4.2 \times 10^{-12}$ . In addition, her father has a rare form of leukaemia that has been also related to exposure of ionizing radiation.

#### E-P13.08

#### Cytogenetically visible copy number variations (CG-CNVs) in banding and molecular cytogenetics of human: about heteromorphisms and euchromatic variants

T. Liehr;

(1) Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany.

Copy number variations (CNVs) having no (obvious) clinical effects have been rediscovered as major part of human genome in 2004. However, for every cytogeneticist microscopically visible harmless CNVs (CG-CNVs) are well known since decades. Harmless CG-CNVs can be present as heterochromatic or even as euchromatic variants in clinically healthy persons. Here I provide a review on what is known today on the still too little studied harmless human CG-CNVs, point out which can be mixed up with clinically relevant pathological CG-CNVs and shortly discuss that the artificial separation of euchromatic submicroscopic CNVs (MG-CNVs) and euchromatic CG-CNVs is no longer timely. Overall, neither so-called harmless heterochromatic nor so-called harmless euchromatic CG-CNVs are considered enough in evaluation of routine cytogenetic analysis and reporting. This holds especially true when bearing in mind the so-called two-hit model suggesting that combination of per se harmless CNVs may lead to clinical aberrations if they are present together in one patient.

## E-P13.12

**Mixed gonadal dysgenesis: cytogenetic and phenotypic findings of two cases with ambiguous genitalia**E. Gokpinar<sup>1</sup>, C. D. Durmaz<sup>1</sup>, Z. Siklar<sup>2</sup>, M. Berberoglu<sup>2</sup>, H. Ilgin-Ruhi<sup>1</sup>;<sup>1</sup>Ankara University Medical Faculty, Department of Medical Genetics, Ankara, Turkey,<sup>2</sup>Ankara University Medical Faculty, Department of Pediatric Endocrinology, Ankara, Turkey.

Mixed gonadal dysgenesis (MGD) is a sex chromosome disorder of sexual development that typically has a mosaic 45,X/46,XY karyotype, with a wide clinical spectrum from Turner-like phenotype to male with infertility. Here, we present two MGD cases that were born with ambiguous genitalia, announced as male. First case was two months old, had 2.3 cm phallus, bifid scrotum, chorde and perineoscrotal hypospadias. Only, left gonad was palpable, and ultrasound showed right inguinal hernia and atrophic right gonad. Karyotype analysis showed 45,X/46,XY mosaicism. We examined peripheral blood and buccal smear cells by interphase FISH for the evaluation of mosaicism. The rate of 45, X was high, 85% and 64%, respectively. Second case was seven months old, had no palpable gonads with 2 cm phallus and one urethral hiatus. Uterus and hypoechoic gonadal structure on the left were shown with USG, but right gonad was not seen. He also had the bicuspid aortic valve. Karyotype analysis was mos 45,X/47,XYY, 95% and 5%, respectively. FISH on buccal smear cells (BSC) and urine sediment (USC) cells were performed to assess mosaicism. Three clones were found in both tissues; X/XY/XY (93%/4%/3% in BSC and 82%/16%/2% in USC). Patients with MGD require multidisciplinary approach for gender assessment and assignment. Our patients were decided to be raised as males.

Reference: Öcal, Gönül, et al. „The clinical and genetic heterogeneity of mixed gonadal dysgenesis: does “disorders of sexual development (DSD)” classification based on new Chicago consensus cover all sex chromosome DSD?“ European journal of pediatrics 171.10 (2012): 1497-1502.

## E-P13.14

**Rare chromosomal aberrations in Iranian patients with Recurrent Pregnancy Loss**V. Yassaei<sup>1,2</sup>, S. Kamalbeik<sup>1</sup>, B. Sadeghi<sup>1</sup>, F. Hashemi-Gorji<sup>1</sup>, S. Hosseini<sup>1</sup>, S. Karimi<sup>1</sup>;<sup>1</sup>Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of; <sup>2</sup>Dept. of Medical Genetic, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of.

There are many factors contributing to Recurrent Pregnancy Loss (RPL). Some cases are diagnosable, preventive and curable; in many cases etiology remains unknown.

We examined blood specimens derived from over 200 couples diagnosed with RPL who referred to our center for genetic testing during 2012 to 2014. Here, we report three RPL cases with negative family history of RPL, normal clinical examination, normal Thrombophilic genetic testing, normal Hormonal and Spermogram analysis and rare chromosomal aberrations.

A non-consanguineous couple (female 37 and male 39 years old) with history of RPL, presented to our center. The female experienced four time abortion, three unsuccessful IVF and two unproductive IUI. Cytogenetic study ascertained 46,XY,t(1;9)(q11;q11) and 46, XX, normal female. These chromosomal regions have significant impact on zygote formation as well as recurrent abortions. In addition, this result has not been previously reported in any couple with recurrent abortions.

A consanguineous couple (female 34 and male 34 years old) with a history of three time first trimester abortion were investigated. Cytogenetic study identified 46,XY,t(6;18)(q16;q11) and 46, XX, normal female.

A non-consanguineous couple (female 34 and male 33 years old) with a history of four time first trimester abortion were examined. Cytogenetic study discovered 46,XX,t(5;19)(p12;p12) and 46, XY, normal male.

Our results suggest that chromosomal abnormalities could be one of the most important causes of RPL. Therefore, cytogenetic study of couples who suffer from recurrent abortion may prevent unnecessary treatment in which chromosomal aberration is the main cause of the disease.

## E-P13.17

**Familial X chromosome translocation, Xq triplication and SHOX gene deletion with short stature; Conflicting results of QF-PCR analysis for Xq segmental triplication**

F. Silan, M. Urfali, O. Yildiz, B. Paksoy, A. Uludag, O. Ozdemir;

Department of Medical Genetics, Canakkale, Turkey.

Objective: SHOX gene deletions cause short stature and related Leri Weill dyschondrosteosis(LWD), Turner syndrome, and disproportionate short stature (DSS)(3-15%). Proband and daughter with SHOX gene deletion which referred for short stature. We aimed to present SHOX gene deletion

with Xq triplication.

Methods: Double blind automated GTG banded karyotype analyses were made from mother and her daughter and diagnosed same translocation. SHOX gene deletion was determined with FISH (Euroclone). QF-PCR (An-eufast) and MLPA(P245, MRCHolland) techniques were used for identification of the the long arm of X chromosome.

Results: 46,XX,trp(X)(q11.2qter)(Xqter→Xq11.2::Xp22.32→Xqter) karyotype and SHOX deletion was detected both proband and daughter. Grandparents have no derived X chromosome, so it was proved de novo translocation and SHOX gene deletion at proband and inherited to daughter. QF PCR shows different ratios for same duplicated region at mother and daughter, DXS8377 marker (Xq28) shows 2.10: 1 ratio at mother and 1.33:1 at daughter.

MLPA shows MECP2 (Xq28) triplication but none of the cases have neurological symptoms.

Conclusions: SHOX gene mutations is related with short stature and made lung like deformity (wrist pain, deviation of radius, cubitus valgus. Proband has also recurrent pregnancy loss. Habituel abortion can be caused by interchromosomal effect of Xq translocation. Male lethality can be possible if only translocated X chromosome inherited from male fetus, because contiguous gene deletions near SHOX gene is also possible. Cut off level for triplications is 1.7:1 at QF PCR but our proband shows 2.10:1 and second case shows 1.33:1 ratio for same triplicated region.

**E-P14.01 New diagnostic approaches, technical aspects & quality control**

## E-P14.01

**Molecular characterization of AIPL1 gene region in the Iranian population: Application of new informative haplotypes**

A. Karimi Moghadam, S. Vallian;

University of Isfahan, Isfahan, Iran, Islamic Republic of.

Leber's congenital amaurosis (LCA) is considered as one of the main causes of congenital blindness. In view of the genetically heterogeneous nature of the disease, indirect diagnosis using comprehensive linkage analysis has proven to be useful in molecular diagnosis procedure. Mutations in AIPL1 gene are one of the leading causes of LCA. In the present study, the application of three single nucleotide polymorphic (SNP) markers related to this region, including rs7212734, rs11658369 and rs8066853 was evaluated for the first time in the Iranian population. The markers were genotyped using tetra-primer ARMS PCR in 154 unrelated healthy individuals. Haplotype frequency and other characteristics of the markers were examined by using the GENEPOL and PowerMarker software. The data indicated the presence of 6 different haplotypes in the Iranian population. Among them, three haplotypes showed highly informative with frequencies  $\geq 0.05$ . Among the informative haplotypes, T-A-A haplotype showed linkage to W278X mutation. Prenatal diagnosis using the markers led to the successful prediction of the fetus genotypes in the all at risk pregnancies and showed that T-A-A haplotype (rs7212734/ rs11658369 / rs8066853) could be considered as informative haplotype for linkage analysis in carrier detection and molecular diagnosis of LCA in the Iranian population.

## E-P14.03

**Determining and Managing Fetal Radiation Dose from Diagnostic Radiology Procedures in Turkey**

M. Demir, I. Cavdar, A. Tuten, H. Ulucan, S. Mehmet; Istanbul University, Istanbul, Turkey.

Objective: We intended to calculate approximate fetal doses in pregnant women who underwent diagnostic radiology procedures and to evaluate the safety of their pregnancies.

Materials and Methods: Fetal radiation exposure was calculated for 304 cases in 218 pregnant women with gestational ages ranging from 5 days to 19 weeks, 2 days. FetDose software (ver. 4.0) was used in fetal dose calculations for radiographic and computed tomography (CT) procedures. The body was divided into three zones according to distance from the fetus. The first zone consisted of the head area, the lower extremities below the knee, and the upper extremities; the second consisted of the cervicothoracic region and upper thighs; and the third consisted of the abdominopelvic area. Fetal doses from radiologic procedures between zones were compared using the Kruskal-Wallis test and a Bonferroni-corrected Mann-Whitney U-test.

Results: The average fetal doses from radiography and CT in the first zone were  $0.05 \pm 0.01$  mGy and  $0.81 \pm 0.04$  mGy, respectively;  $0.21 \pm 0.05$  mGy and  $1.77 \pm 0.22$  mGy, respectively, in the second zone; and  $6.42 \pm 0.82$  mGy

and  $22.94 \pm 1.28$  mGy, respectively, in the third zone ( $p < 0.001$ ). Our results showed that fetal radiation exposures in our group of pregnant women did not reach the level (50 mGy) that is known to increase risk for congenital anomalies.

Conclusion: Fetal radiation exposure in the diagnostic radiology procedures in our study did not reach risk levels that might have indicated abortion.

#### E-P14.04

##### Evaluating engraftment success by determining the proportion of donor and recipient interphase cells present in sex-mismatched bone marrow transplantation

*L. Malee, V. Korkiatasakul, P. Phornsarayuth, T. Chareonsirisuthigul, B. Rerkamnuaychok; Human Genetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*

Introduction: Bone marrow transplantation (BMT) continues to be an important treatment for patients with malignant hematologic disorders and thalassemia. The assessment of chimerism, the ratio of donor to recipient cells, is important for monitoring the engraftment of donor cells and determining the recurrence of the original disease after BMT. The aim of this study is to monitor the proportion of host and recipient cells in sex-mismatched BMT using interphase fluorescence in situ hybridization (FISH).

Materials and Methods: A total of 856 bone marrow or peripheral blood specimens were received from 170 BMT patients which were HLA-matched or partially mismatched, but had sex-mismatched donors at Ramathibodi hospital between January 2011 and December 2015. Interphase FISH analysis using dual color X/Y probes with 500 interphase nuclei were scored.

Results: A total of 170 cases were diagnosed as malignant hematologic disorders (n=147), thalassemia (n=15), anemia (n=3) and others, such as Gaucher disease, neuroblastoma, adrenoleukodystrophy (n=5). These patients were divided into three age groups (1 to 20, 21 to 40 and 41 to 60 years).

Patients from 1 to 20 age group had the most completed chimerism in post-BMT (123 of 137 cases, 89.78%). However, patients aged over 40 years old had the lowest complete chimerism (6 of 14 cases, 42.68%).

Conclusions: Our results suggest that young age group has the highest successful rate of BMT. FISH studies can be used to detect chimerism for XX and XY cells for evaluating successful engraftment post-BMT status.

#### E-P14.05

##### Evaluating critical performance factors between three different instruments for HRM genotyping assays

*L. Papazisi, L. Jiang, R. W. Weick, F. Somanji, Z. Kabir, J. J. Huusonen, I. T. Knight; Canon U.S. Life Sciences, Inc., Rockville, MD, United States.*

Introduction: PCR followed by high-resolution melting (HRM) is a fast, reliable, and inexpensive method to perform DNA genotyping. Multiple manufacturers have added HRM capabilities to their thermocyclers. The HRM-based Novallele™ genotyping assays (Canon BioMedical) for research were developed using the CFX384 Touch™ (Bio-Rad). Our data demonstrate that carefully designed and optimized HRM assays are easily transferred between different thermocyclers, expanding the utility of these assays and HRM analysis as a method.

Materials and methods: We compared the performance of six small amplicon and six unlabeled probe Novallele genotyping assays using three different HRM-enabled thermocyclers. Assay performance, including PCR robustness and genotyping reliability, was determined for the CFX384 Touch, LightCycler® 480 (Roche) and Rotor-Gene® Q (QIAGEN®) instruments. Both genomic DNA and synthetic constructs were tested, and the data were analyzed using the HRM analysis software for each respective instrument. Results: All 12 assays were able to detect mutations using the different instruments. The PCR amplification and melting temperature difference between the normal and mutated amplicons was maintained across the instruments. For four of the assays, minor optimization of either an annealing temperature by  $\leq 1.5^\circ\text{C}$  or a change of cycle number by +5 cycles resulted in slightly improved performance. Most importantly, reliable genotyping was achieved for these assays using a wide range of starting material, approximately 6-60 ng.

Conclusion: Novallele genotyping assays perform robustly using different HRM-enabled thermocyclers, which demonstrates the successful assay design. With no or minimal optimization for each instrument, the assays can reliably genotype various targets.

#### E-P14.07

##### Efficiency of NimbleGen-based target capture for small gene panels

*M. Belenikin<sup>1,2,3</sup>, T. Meshcheryakova<sup>4</sup>, S. Zhilina<sup>4</sup>, G. Mutovin<sup>1</sup>;*

*<sup>1</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation,*

*<sup>2</sup>Lomonosov Moscow State University, Moscow, Russian Federation, <sup>3</sup>Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russian Federation, <sup>4</sup>Research Center for Children Medical Care, Moscow, Russian Federation.*

Background: Choice of target-enrichment strategy for NGS is important for design of target panels and results of sequencing. The aims of study: Testing and analysis of NimbleGen-based gene capture strategy for use with small gene panels. Methods: NimbleGen SeqCap system for targeted genomic enrichment (2-stage hybridization procedure for effective work with gene panels of small size). Results: The designed gene panel covers 34 genes (total size of exone regions  $\sim 100$ K bp); distribution of genes on the chromosomes was as follows: one gene is located on every chromosomes 1, 3-8, 12-15, 18, 20; two genes are located on every chromosomes 9, 11, 16; three genes are located on chromosome 19, six genes are located on every chromosomes 2 and X. We sequenced 100 samples; during every runs we studied up to 12 samples. A number of reads (454 GSJunior), relating to every chromosomes after sequencing were as follows (are listed only four mostly covered chromosomes): 28.5-49.3% on chromosome 2 (we vary a number of experimental parameters), 7.6-15.7% on chromosome 9, 5.9-12% on chromosome X, 3-7.6% on chromosome 16. Conclusions: Owing to features of NimbleGen probe design NimbleGen target-enrichment strategy is not optimal for the design of small gene panels (total size of exone regions  $\sim 100$ K bp), especially for genes with very different numbers of exons. So, for small gene panels the using of other target-enrichment strategies producing more uniform probe coverage is more suitable.

#### E-P14.08

##### WAVE/DHPLC technology as a promising platform for diagnostics of diseases with distinct clinical manifestation caused by contiguous genes

*A. N. Voitovich<sup>1</sup>, I. Kirsanov<sup>1</sup>, T. Urzhumtseva<sup>1</sup>, V. Larionova<sup>2</sup>, K. Isaeva<sup>1</sup>;*

*<sup>1</sup>Academy of Molecular Medicine, Saint-Petersburg, Russian Federation, <sup>2</sup>Center For Rare Diseases and Molecular medicine „IEM“ RAS, Saint-Petersburg, Russian Federation.*

Neurofibromatosis (NF) and polycystic kidney disease are the most common autosomal dominant genetic disorders, with a frequency of 1: 3500 and 1:400 newborns respectively. Both of them are readily recognizable by clinical and clinical instrumental methods. For example, the gene of neurofibromine encompasses 61 exons. 50% of the mutations occur de novo. Problems of detection of the mutations in NF1 gene are caused by its large size, a lack of the major mutation in European populations, as well as by an interference with pseudogenes with alike structure but not function.

The modern methods developed for genetic diseases diagnostics may have different diagnostic and economic value at every particular case. The "Gold Standard" of the genetic diagnosis, ie Sanger sequencing, does not meet modern criteria of high-technology, time-, labor-, and cost-consuming method. To improve its performance, some supportive technology are in use, such as pre-mutation screening methods for selection only those samples in which the mutation is detected.

WAVE technology of mutation screening by denaturing high-pressure chromatography (DHPLC) takes the advantage of exome sequencing and NGS technologies. Exome sequencing does not allow for detection of all mutations in contiguous genes while producing an odd information difficult to be interpreted properly both legally and ethically. NGS being currently expensive itself, requires specialists experienced in bioinformatics. Moreover, all findings detected using that technologies must be verified by Sanger method. WAVE technology is the most appropriate for testing contiguous genes and may be recommended to insurance companies for looking closely at.

#### E-P14.09

##### Lack of amplification in next generation sequencing? Check for deletions

*R. Bayramov, M. Ensar Doğan, M. Cerrah Güneş, K. Korkmaz Bayramov, Y. Ada, Ç. Saatçi, Y. Özkul, M. Dündar;*

*Erciyes University, Kayseri, Turkey.*

Introduction: Modern methods such as Next-Generation Sequencing, leave us face to face with big data that needs specialized interpretation. In some cases, there can be a unique set of circumstances that demand unusual strategy.

Materials and Methods: Blood samples were collected into tubes containing EDTA, from the 31 male patients attended department of Medical Genetics at Erciyes University with a preliminary diagnosis of Duchenne Muscular

Dystrophy. DMD gene next generation sequence analysis was performed by using Illumina MiSeq platform after DNA isolation.

Results: Analysis of 21 patients revealed nonpathogenic variants, in 3 patient c.5637 C>A, c.4455 C>T, and c.10699 C>T hemizygous nonsense mutation variants were observed. But in 10 samples for some exons, the lack of amplification were detected. For this phenomenon, there can be several explanations. But first we decided to check for deletions by Multiplex Ligation-dependent Probe Amplification method. In the exactly same exons deletions were determined in all samples (100%). Because of a small number of cases we obtained differently but similar results described in the literature which is 90%-95%.

Conclusions: In interpretation of this experience we can say that in male patients during sequence analysis of X-linked dominant diseases such as X-Linked Hypophosphatemia, Rett syndrome, Aicardi syndrome and X-linked recessive diseases such as Hemophilia A, B, X-linked ichthyosis, X-linked agammaglobulinemia and etc. diseases which has in etiopathogenesis sequence changes and deletions in responsible genes, lack of amplification should be considered as a deletion and confirmed by adequate methods.

#### E-P14.10

##### Five years of experience from the new technological units in the Hospital Sant Pau: nucleic acid extraction and sequencing.

E. Martínez-Sánchez<sup>1</sup>, S. Quero<sup>1</sup>, M. Baena<sup>2</sup>, M. Garf<sup>1</sup>, A. Lasq<sup>2,3</sup>,

<sup>1</sup>Laboratorios Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>2</sup>Genetic Department. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>U-705, CIBERER, Barcelona, Spain.

#### INTRODUCTION

Since 2011, the laboratories of the Hospital de la Santa Creu i Sant Pau have incorporated two new technological units which are available to scientists from our hospital. The aim of this project is to provide scientists with infrastructure to effectively carry out molecular diagnostic tests. Currently the core facilities are oriented on these areas: automatic nucleic acid extraction, capillary and Next Generation sequencing (NGS).

#### MATERIAL AND METHODS

Each technological unit has the following equipment:

- DNA extraction area: Autopure (Qiagen), Magnapure (Roche)
- Sequencing area: MiSeq (Illumina) for massively parallel sequencing, and the 3500Dx and 3500XL Dx Genetic Analyzer (Applied Biosystems) for capillary sequencing.

The laboratory technician performs sample collection, preparation and manipulation procedures according to Standard Operating Procedures.

#### RESULTS

The following table shows the number of processed samples in both areas

Year	Results		
	DNA extraction (nº samples)	Capillary sequencing (nº samples)	NGS sequencing (nº samples)
2011	4063	38262	0
2012	5217	44032	0
2013	4228	36041	0
2014	4732	29908	366
2015	4786	28729	1280

#### CONCLUSIONS

The core facility described here, is a centralized, shared resource that provides clinic and laboratory investigators services and expert consultation. Next generation sequencing analysis approach allowed us to study more genes with a significant cost saving and a higher sequencing accuracy. We have also obtained a shorter turnaround time allowing a quicker molecular diagnosis and covering the demand of clinicians and patients.

#### E-P14.11

##### Benefit of using a commercial kit for prenatal non invasive testing : fetal RHD genotyping with kit jacques boy

E. Guinchard, S. Monnier, C. Marthinet;

EFS Rhône-Alpes site de Lyon-GHE, 69677 BRON Cedex, France.

Background: fetal RHD genotyping is an innovative and non-invasive method to assess the risk of fetus's hemolytic disease of anti-D allo-immunized pregnant woman.

Patient/method: the commercial kit J Boy® for genotyping has been tested in the laboratory of Lyon-GHE: exon 5, 7, and 10 are amplified real time by PCR using TQMan technology.

A maize's DNA is included as internal control.

The most important parameters of the method (both sensibility and the specificity) have been tested, checked and verified: the negative predictive value, detection of new variant forms of the RHD gene. Genotyping results of

pregnant women have been compared with RH1 phenotype at birth. The correlation study has allowed to establish the interpretation criteria. The residual risk analysis has allowed to understand the actual limitation of the method due to the low concentration of fetal DNA in the maternal plasma and to define criteria of biological interpretation: the negative result must be confirmed by getting the same result on another sample a few weeks later.

#### Conclusion:

This commercial kit very easy to use but several samples must be analysed simultaneously to reduce the unit cost for each test.

#### E-P14.12

##### Performance of the Agilent D5000 and High Sensitivity D5000 ScreenTape Assays for the Agilent 4200 TapeStation System

R. Nitsche<sup>1</sup>, L. Prieto-Lafuente<sup>2</sup>, C. MacDonald<sup>2</sup>, E. Brown<sup>2</sup>;

<sup>1</sup>Agilent Technologies, Waldbronn, Germany, <sup>2</sup>Agilent Technologies, Edinburgh, United Kingdom.

The new Agilent 4200 TapeStation system provides automated, fast, and reliable DNA and RNA electrophoresis for up to 96 samples using prepackaged reagents and minimal manual handling. The Agilent D5000 ScreenTape and High Sensitivity D5000 ScreenTape assays have been developed for the separation and analysis of DNA fragments from 100 bp to 5,000 bp, a size range that complements and resides between the Agilent D1000

ScreenTape and the Genomic DNA ScreenTape assays. The 4200 TapeStation system and the DNA ScreenTape assays can be used at several steps of the Next Generation Sequencing (NGS) workflow. With the emergence of methodologies, such as the use of Transposomes, NGS library sizes are tending to increase beyond 1,000 bp, even for the short-read NGS technologies. This Poster focuses on the performance of both D5000 ScreenTape assays with respect to the accuracy and precision of quantification and sizing, as well as the sensitivity of these assays. Data analysis for quantification and molarity determination was compared against the corresponding assay for the Agilent 2100 Bioanalyzer system. Additionally, performance of both the D5000 and High Sensitivity D5000 assays on the 4200 TapeStation was compared to the 2200 TapeStation system.

#### E-P14.13

##### Use of saliva and salivary DNA for comprehensive genotyping based on RealFast and StripAssays

A. Berndt, B. Hauser, C. Oberkanins, H. Puehringer; ViennaLab Diagnostics, Vienna, Austria.

Nucleic acids extracted from blood are the most common sample types used in molecular genetic diagnostics. Blood is an abundant and reliable specimen, yielding high amounts of DNA and RNA with homogenous quality. Since drawing blood is an invasive procedure, and DNA extraction is time consuming and requires special equipment, salivary DNA and saliva direct-to-PCR represent promising alternative approaches in clinical diagnostics. Saliva is considered to represent an ultra-filtrate of blood, and thus contains in abundance a huge variety of diagnostically valuable molecules. Apart from being known to contain polymerase chain reaction (PCR) inhibitors, saliva is mucous and inhomogenous and thus often difficult to handle. Moreover, its human DNA content is by far lower compared to blood. Here we examined blood-derived DNA, salivary DNA and saliva direct-to-PCR to carry out reliable genotyping based on single-plex RealFast and multi-plex StripAssays for up to 22 mutations. Our data suggest that saliva collected and stored in dedicated devices is suitable to be used for isolating ample amounts of high-quality DNA, but also for direct-to-PCR approaches towards comprehensive molecular diagnostics.

#### E-P14.14

##### Evaluating the association of IL2RA and CTLA4 genes polymorphisms with type I diabetes mellitus in children of the northwest of Iran

M. Ranjouri<sup>1</sup>, P. Aob<sup>1</sup>, M. Saber<sup>2</sup>, A. Ramezani<sup>1</sup>;

<sup>1</sup>Zanjan University of Medical Science, Zanjan, Iran, Islamic Republic of, <sup>2</sup>Tehran University of Medical Science, Tehran, Iran, Islamic Republic of.

Introduction: Type-1 diabetes (T1D) is caused by autoimmune-mediated destruction of insulin-secreting cells, and a variety of genetic predisposing and environmental factors are known to influence its pathogenesis. The aim of this study was to investigate the association of CTLA4 and IL2RA polymorphisms with type 1 diabetes in children of northwest of Iran.

Material and Methods: total genomic DNA was extracted by salting-out from

peripheral blood of 50 T1D patients and 50 controls. PCR and direct sequencing were used for genotyping CTLA4 (exon1) and IL2RA (intron1) genes in patients and controls.

Results: the frequency of G allele and GG genotype of the CTLA-4 (+49A/G) polymorphism in patients were significantly different from that of controls (26% vs. 11%,  $p = 0.006$ ). Moreover, the allele frequency of the new SNP identified in exon1 of CTLA4 was significantly different was between patients and controls (14% vs. 3%,  $p = 0.006$ ). These results suggest that the GG genotype of +49 A/G is associated with hyperglycemia in the patients ( $p = 0.0067$ ). However, no significant difference was observed in the frequency of IL2RA (ss52580101C/A) polymorphism between patients and controls (2% vs. 4%,  $p = 0.41$ ).

Conclusions: The results support the association of T1D and CTLA4 +49 A/G SNP in the studied population. However, no significant relationship between IL2RA ss52580101C/A polymorphism and T1D was observed.

## E-P15 Personalized/Predictive Medicine and Pharmacogenomics

### E-P15.06

#### CYP2D6 allele distribution among Macedonian, Albanian and Romani population living in the Republic of Macedonia

M. Kuzmanovska, M. Dimiskovska, I. Maleva Kostovska, P. Noveski, E. Sukarova Stefanovska, D. Plaseska-Karanfilска;

Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", Macedonian Academy of Sciences and Arts, Skopje, Macedonia, The Former Yugoslav Republic of.

Cytochrome P450 2D6 (CYP2D6) is an enzyme of great importance for the metabolism of clinically used drugs. The CYP2D6 gene is highly polymorphic and has the most variations among the CYP450 gene superfamily, with more than 100 variants identified so far.

The aim of this study was to investigate the allele distribution of CYP2D6 variants in the Macedonian, Albanian and Romani population. A total of 300 unrelated individuals, 100 individuals from each ethnic group, were genotyped using long range PCR and multiplex single base extension (SNaPshot) method.

The most frequent variants and equally distributed in the three analyzed ethnic groups were the fully functional alleles \*1 and \*2. The most common non functional allele in all ethnic groups was \*4, which reached the highest frequency among Albanians (22.5%) and the most common allele with decreased activity was \*41, which was more frequent among Romani (23%) when compared to Macedonians (11%,  $p=0.002$ ) and Albanians (10.5%,  $p=0.0012$ ).

Among Albanians the percentage of poor metabolizers was 7%, 6% among Romani and 4% among Macedonians. Higher percentage of ultrarapid metabolizers (UMs) was observed among Macedonians (5%), when compared to the Albanians and Romani (1% in each group).

In conclusion, our study showed that CYP2D6 gene locus is highly heterogeneous in the three examined ethnic groups. The prevalence of CYP2D6 allelic variants and genotypes in Macedonia is in accordance with the other European populations.

### E-P15.08

#### Cytochrome P450 2D6 (CYP2D6) polymorphisms in the drug abused postmortem subjects from South Turkey

S. Kocaturk-Sel<sup>1</sup>, N. Daglioglu<sup>2</sup>, P. Efeoglu Ozseker<sup>2</sup>, H. Oksuz<sup>1</sup>, A. Eren<sup>3</sup>, M. Gulmen<sup>2</sup>, H. Luleyap<sup>1</sup>, M. Yilmaz<sup>1</sup>;

<sup>1</sup>University of Cukurova Faculty of Medicine, Department of Medical Biology, Adana, Turkey, <sup>2</sup>University of Cukurova Faculty of Medicine, Department of Forensic Medicine, Adana, Turkey, <sup>3</sup>Council of Forensic Medicine, Adana, Turkey.

Introduction: Most patient populations show large inter-individual variability with respect to drug response and toxicity. This variation can be of physiological, pathophysiological, environmental or genetic origin. Cytochrome P450 2D6 (CYP2D6) has high relevance in drug metabolism. CYP2D6 is involved in the metabolism of 20-25% of clinically used drugs and exhibits a clinically relevant gene polymorphism that modifies the pharmacokinetics of nearly 10% of all drugs. Opiate addiction is one of the most important issues of drug dependence in the forensic toxicology in the world. Most of drug over dose related deaths are caused by opioids such as morphine, codeine, dextromethorphan, ethylmorphine and tramadol. Interestingly, an opiate addict with impaired CYP2D6 enzyme may show unexpected adverse drug reactions towards regular doses compared to controls due to poor metabolism of drugs.

**Materials and Methods:** In this study, polymorphisms of drug abused post-mortem samples and respected controls; CYP2D6\*3 (n=38), CYP2D6\*4 (n=49), and CYP2D6\*6 (n=48) single nucleotide polymorphisms (SNPs) have analyzed with TagMan drug metabolism genotyping assay kit by Real-Time PCR.

**Results:** The genotype frequencies of CYP2D6\*3, CYP2D6\*4 and CYP2D6\*6 yielded no significant results for both post mortem subjects compared to control groups.

**Conclusion:** Between people with different ethnic backgrounds the pattern of CYP2D6 polymorphisms and phenotypes differs dramatically. Although we did not obtain a significant result from our data, such studies should be done with larger sample sizes.

This study has been supported by Cukurova Research Fund (Adana, Turkey).

### E-P15.09

#### Pharmacogenetics as a complementary tool of medication therapy management to improve patient's outcomes

M. P. Ribate, L. Sáez-Benito, N. Berenguer, A. Sáez-Benito, C. B. Garcia; San Jorge University, Villanueva de Gállego, Spain.

**INTRODUCTION:** At present a high percentage of the pharmacological treatments do not reach the wished benefits, due to the generation of adverse effects or to the lack of effectiveness. This occurs especially in polypharmacy patients. Medication Therapy Management services and pharmacogenetic approach can help to solve and prevent these problems.

**MATERIALS AND METHODS:** We present two kidney-transplanted males treated with Tacrolimus as immunosuppressant therapy and with Omeprazole as gastrointestinal prevention. Analyzing results of Medication Therapy Management services, one of them (patient 1) showed fluctuating Tacrolimus levels, gastrointestinal complications and muscular pain. The existence of an interaction between both drugs was suspected.

The principal genes involved in the transport and metabolism of these drugs are ABCB1, CYP3A4, CYP3A5, CYP2C19 and POR, and were studied by PCR-RFLP.

**RESULTS:** Genotyping results are shown in the table.

A change in antiacid therapy was proposed as solution (substitution of Omeprazole for Rabeprazole), for which there is evidence of lower incidence in its metabolism profile.

**CONCLUSIONS:** Pharmacogenetics and Medication Therapy Management services are raising fields that are becoming essential in the pharmaceutical area. Both of them seek to improve drug therapies effectiveness and toxicological effects. The substitution of Omeprazole for Rabeprazole stabilized plasmatic concentrations of Tacrolimus and decreased adverse effects in the patient.

Genotyping results				
	Patient 1		Patient 2	
	Genotype	Phenotype	Genotype	Pheno-type Inter- mediate metab- olizer
CYP2C19*2	*2/*2	Poor metabolizer	/*2	
CYP2C19*3	Missing	-	Missing	-
ABCB1	TT	↓ protein expression	CC	wild-type
CYP3A4	AG	Dose adjustment	AA	wild-type
CYP3A5	*1/*1	wild-type	*1/*1	wild-type
POR*28	CC	wild-type	CC	wild-type

### E-P15.11

#### Pharmacogenetic analysis of CYP genes, VKORC1, UGT1A1 and MDR1 at West Anatolia Region

C. Silan<sup>1</sup>, O. Yıldız<sup>2</sup>, O. Ozdemir<sup>2</sup>, F. Silan<sup>2</sup>;

<sup>1</sup>Department of Pharmacology, Faculty of Medicine, Canakkale, Turkey, <sup>2</sup>Department of Medical Genetics, Canakkale, Turkey.

Pharmacogenetic analysis of some genes have clinical importance. CYP2C9, CYP2C19 and CYP2D6 are most frequently tested genes. VKORC1 analysis also indicated with CYP2C9. Varfarin using patients for accurate dosing and protecting patients from complications. UGT1A1 gene poor metabolizer patients have high risk for life-threatening complications of Irinotecan and Mikofenolate Mofetil. CYP2C19 analysis performed because slow metabolizer patients are high risk for ineffective treatment with Clopidogrel and second stroke. CYP2D6 genotyping also indicated for

We present our genotype results from patients tested for pharmacogenetic analysis because of using drugs with narrow therapeutic index and/

or high risk for life-threatening complications. Analysis performed from EDTA blood samples, DNA isolated spin column method and real-time PCR method (LightCycler2.0). Total 317 patients tested for 6 genes. Genotyping results showed at Table.

	Wild n/%	Heterozy- gous n/%	Mutant n/%	Total n
<b>VKORC1 (C1173T)</b>	34/20,3	79/47,4	54/32,3	167
<b>VKORC1 (G1639A)</b>	43/25,7	80/47,9	44/26,4	167
<b>CYP2C9 2*</b>	145/81,9	29/16,4	3/1,7	177
<b>CYP2C9 3*</b>	70/	100/	7/	177
<b>CYP2C19 2*</b>	57/62,7	30/32,9	4/4,4	91
<b>CYP2C19 3*</b>	90/98,9	0/0	1/1,1	91
<b>CYP2D6</b>	17/89,5	2/10,5	0	19
<b>UGT1A1 28*</b>	2/20	<b>3/30</b>	<b>5/50</b>	10
<b>MDR1(C3435T)</b>	11/40,7	11/40,7	5/18,6	27

Total Case:324

High frequency of UGT1A1 poor metaboliser genotypes indicated the importance of clinical pharmacogenetics.

#### E-P15.12

#### VKORC1 and CYP2C9 genotypes in patients with pulmonary thromboembolism

**A. Zamani<sup>1</sup>, A. G. Zamani<sup>2</sup>, S. Yosunkaya<sup>1</sup>, M. S. Yıldırım<sup>2</sup>**

<sup>1</sup>Necmettin Erbakan University, Meram Medical Faculty, Department of Pulmonary Diseases, Konya, Turkey, <sup>2</sup>Necmettin Erbakan University, Meram Medical Faculty, Department of Medical Genetics, Konya, Turkey.

**Background:** Warfarin is the most widely used anticoagulant with a narrow therapeutic index. The activity and metabolism of warfarin are regulated by vitamin K epoxide reductase complex subunit 1 (VKORC1) and cytochrome P450-2C9 (CYP2C9) genes. Genetic variability of warfarin dosing has been attributed to polymorphisms in these genes.

**Objective:** To investigate the impact of genetic factors on warfarin dosage requirement in patients with pulmonary thromboembolism (PTE) at the Pulmonary Diseases Department.

**Methods:** The VKORC1 G1639A, C1173T and CYP2C9(\*1/\*2/\*3) allelic variants were identified by using real time-PCR in 17 (8 males and 9 females) patients with PTE.

**Results:** Only 4 patients were found to be normal for VKORC1 mutations, while 3 of them were heterozygous and one was compound heterozygous for CYP2C9. Eight of the remaining 13 patients were homozygous and the other 5 were compound heterozygous for both mutations. CYP2C9 gene was found normal in 4 patients (\*1\*1), heterozygous mutant in 9 patients (\*1\*3), compound heterozygous in 2 patients (\*2\*3) and homozygous mutant in 2 patients (\*3\*3). There were no wild-type genotype for mutations in both genes of patients. Warfarin dose was adjusted based on genetic testing results of the patients.

**Conclusion:** VKORC1 and CYP2C9 genotypes can be used to help determine the optimal starting dose of warfarin for patients with PTE. Warfarin dose management should be personalized on the basis of a patient's genetic profile.

#### E-P15.14

#### Influence of genetic polymorphisms in the anti-TB drug-induced hepatotoxicity in an indigenous and non-indigenous population from Brazil

**V. M. Zembrzuski<sup>1</sup>, D. R. Andrade<sup>2</sup>, K. T. S. Moraes<sup>2</sup>, M. M. Heinrich<sup>1</sup>, R. L. F. Teixeira<sup>1</sup>, P. H. C. Acero<sup>1</sup>, G. M. Cunha<sup>1</sup>, R. Souza-Santos<sup>1</sup>, J. H. R. Croda<sup>2</sup>, P. C. Basta<sup>1</sup>**

<sup>1</sup>Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, <sup>2</sup>Universidade do Grande Rio, Duque de Caxias, Brazil, <sup>3</sup>Oswaldo Cruz Foundation, Campo Grande, Brazil.

**Introduction:** First-line anti-tuberculosis (TB) drugs are responsible for the occurrence of several adverse drug reactions (ADR). Hepatotoxicity is one of most serious ADR and appears soon in the beginning of treatment. Although already known the main mechanisms involved in the development of hepatotoxicity, in Brazil this field is little explored, especially in vulnerable populations. Therefore, the objective of this study was to estimate the incidence

of hepatotoxicity during TB treatment and investigate the associations with genetic polymorphisms, clinical and epidemiological factors, comparing indigenous and non-indigenous patients.

**Material and Methods:** Clinical and epidemiological variables, serum levels of liver enzymes and polymorphisms of the genes NAT2, CYP2E1 and GSTM1 were investigated. Non-conditional logistic regression was used to identify factors associated to hepatotoxicity. Odds ratio was used as association measure.

**Results:** The incidence of hepatotoxicity was 19.7% for all patients, being 50% more frequent in indigenous (p=0.055). We identified 10 SNPs previously described and, in indigenous, one new nonsynonymous SNP of NAT2. 54.6% of patients expressed slow acetylation phenotype profile. The frequency of null genotype of GSTM1 was higher in non-indigenous (p=0.002) while no significant differences in relation to polymorphism of CYP2E1 were observed between the groups. Hepatotoxicity was associated to patients older than 60 years old and indigenous (OR=26.0; 95%CI:3.1-217.6; OR=3.8; 95%CI:1.3-11.1, respectively). Furthermore, hepatotoxicity was associated to slow acetylation profile in indigenous patients (OR=10.7; 95%CI:1.2-97.2).

**Conclusions:** Our findings reinforce the importance of develop personalized therapeutic schemes in order to avoid unfavorable outcomes.

#### E-P15.15

#### Distribution of allelic and genotypic frequencies of NAT2 and CYP2E1 variants in Moroccan population

**S. Guoua<sup>1,2</sup>, I. Ratbi<sup>1</sup>, F. Laarabi<sup>2</sup>, S. Elalaoui Chafai<sup>1</sup>, I. Cherkaoui Jaouad<sup>1</sup>, A. Barkat<sup>3</sup>, A. Sefiani<sup>1,2</sup>**

<sup>1</sup>Centre de génomique humaine, Faculté de médecine et de pharmacie, Mohammed V University in Rabat, Morocco, Rabat, Morocco, <sup>2</sup>Institut National d'hygiène, Rabat, Morocco, <sup>3</sup>faculty of medicine and pharmacy rabat, Rabat, Morocco.

**Background:** Several pathogenesis and genetic factors influence predisposition to antituberculosis drug-induced hepatotoxicity (ATDH) especially for isoniazid (INH). However, the major susceptibility genes for ATDH are N-acetyltransferase 2 (NAT2) and cytochrome P450 2E1 (CYP2E1). NAT2 gene determines the individual's acetylator status (fast, intermediate or slow) to metabolize drugs and xenobiotics, while CYP2E1 c1/c1 genotype carriers had an increased risk of ATDH.

Polymorphisms of the NAT2 and CYP2E1 genes vary remarkably among the populations of different ethnic origins.

The aim of this study was to determine, for the first time, the frequency of slow acetylators in Moroccan population by genotyping of NAT2 gene variants and determining the genotype c1/c1 for CYP2E1 gene, in order to predict adverse effects of Tuberculosis treatment, particularly hepatotoxicity.

**Results:** The frequencies of specific NAT2 alleles were 53%, 25%, 2% and 4% for NAT2\*5, NAT2\*6, NAT2\*7 and NAT2\*14 respectively among 163 Moroccan studied group. Genotyping of CYP2E1 gene, by real-time polymerase chain reaction using TaqMan probes, revealed frequencies of 98.5% for c1/c1 and 1.5% for c1/c2 among 130 Moroccan studied group.

**Conclusion:** The most prevalent genotypes of NAT2 gene in Moroccans are those which encode slow acetylation phenotype (72.39%), leading to a high risk of ATDH. Most Moroccans are homozygous for c1 allele of CYP2E1 gene which aggravates hepatotoxicity in slow acetylators.

This genetic background should be taken into account in determining the minimum dose of INH needed to treat Moroccan TB patients, in order to decrease adverse effects.

#### E-P15.16

#### The Impact Of CYP2C9, VKORC1, ORM1,CYP4F2, GGCX, MDR1 Gene Polymorphisms On Warfarin Dosage

**H. Akar<sup>1</sup>, L. Dinc Asarcikli<sup>2</sup>, Y. Tunca<sup>1</sup>**

<sup>1</sup>Gulhane Medical Military Academy, Ankara, Turkey, <sup>2</sup>Dişkapi Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey.

**Introduction:** Warfarin is a widely used anticoagulant that shows a high inter-individual variability in the dose needed to achieve target anticoagulation. The main objective of this study was to identify the genetic and the environmental factors on the variabilities in warfarin dose requirements.

**Methods :** CYP2C9 (rs1057910, rs 1799853), VKORC1 (rs 9923231, rs 9934438), ORM1 (rs 17650) , CYP4F2 (rs 2108622), GGCX (rs2592551), MDR1 (rs1045642) polymorphisms were studied by hybridization probes in 235 patients from Turkey who had stable doses and international normalized ratios of 2-3 at their last three consecutive visits.

**Results:** Patients' age, weight, and height were associated with warfarin maintenance dose. VKORC1-G1639A mt and heterozygote genotype was

associated with a higher maintenance dose compared to those with the wild genotype (41.13mg/week vs.31.32mg/week). VKORC1-C1173T wild genotype was associated with a higher maintenance dose compared to those with mutant and heterozygote genotype (42,79/week vs.30,78/week). CYP2C9 \*3 CC variant was related to lower warfarin dose (12,70 mg/week) requirement compared to AA genotype (35,93 mg/week). CYP2C9 \*2 CC variant was related to higher warfarin dose (35,93 mg/week) requirement compared to TT genotype (32,12 mg/week). ORM1 GG variant was related to lower warfarin dose (27,76 mg/week) requirement compared to AA genotype (37,27 mg/week). There was no difference in warfarin doses with GGCX, MDR1 and CYP4F2 variants.

Conclusion: Age and CYP2C9\*3, ORM1, VKORC1 were the most significant determinants of warfarin dosage in this preliminary study including Turkish patients.

## E-P17 Epigenetics and Gene Regulation

### E-P17.01

#### Analysis of epigenetic changes in apoptotic mechanism triggered by caffeic acid phenethyl ester (CAPE) and DNA methyl transferase inhibitor (DNMT1) zebularine in MDA-MB-231 breast cancer cell line

O. Eroglu, E. Guvenir Celik, M. Celen, E. Karacoban;  
Bilecik Şeyh Edebali University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Bilecik, Turkey.

High dose chemotherapeutic drugs used in cancer treatment researches may have cytotoxic effects. In this study, cytotoxic effects of the drugs were annihilated by the combined use of caffeic acid phenethyl ester (CAPE) and DNA methyl transferase inhibitor (Zebularine). Then, the regulation of genes related to apoptotic pathway in breast cancer was studied epigenetically. MDA-MB-231 breast cancer cell line was grown by culturing of cells. Drug doses were determined by MTT assay. Survival assays showed that the drugs decrease the cell viability. Colony sizes created by the cells were examined by colony formation assay and soft agar colony formation assay and found out that drugs prevent the cell growth. DNA was insulated from cells which were drugged, then bisulphite modification was made and state of methylation of TP53, caspase-9, caspase-8 and caspase-3 genes were analysed by Methylation Specific PCR (MSP). Methylation changes in the aforesaid genes gave significant results. Data obtained in consequence of MSP, in comparison with the control group, methylation changes observed at TP53 50% unmethylation ( $p<0.05$ ), caspase-9 70% unmethylation ( $p<0.001$ ), caspase-8 60% unmethylation ( $p<0.01$ ), and at caspase-3 60% unmethylation ( $p<0.01$ ).

As a result of the findings, it can be stated that cytotoxic effects disappeared after the combined therapy of Zebularin - CAPE. In the study, epigenetically significant results were obtained in the stimulation of the apoptosis through caspases by unmethylating the genes in relation of apoptosis. The findings are highly important in terms of annihilation of the cytotoxic effects through combined therapy and the fight against cancer

### E-P17.02

#### Analysis of the effect of Zebularine on caspase-3, caspase-8 and caspase-9 in SKBR3 breast cancer cell line

O. Eroglu, M. Celen, E. Guvenir Celik;  
Bilecik Şeyh Edebali University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Bilecik, Turkey.

DNMT inhibitor nucleotide analogues can reorganize the expression of the genes related to apoptosis and tumour suppressor genes which are silenced by methylation. Thus, it can trigger apoptosis formation in tumour cell. Zebularine which is DNA methyl transferase inhibitor (cytidine analogue) is one of these agents. This study aimed to analyse the effects of zebularine in SKBR3 breast cancer cell line on cytotoxic and apoptotic mechanism and it is important in the sense that it is the first study on the issue in the literature. SKBR3 cell line used in the study is grown at DMEM medium. In order to evaluate the effects of Zebularine on cytotoxicity, cell viability, cell growth, MTT assay, cell survival assay, soft agar colony formation assay, wound healing assay were done respectively. At the same time, in order to understand the effects on apoptotic mechanism, Methylation Sensitive High Resolution Melting (MS-HRM) was done for the methylation analysis of caspase-3, caspase-8 and caspase-9 genes after bisulphite transformation.

As a result of the findings, it is observed that Zebularine considerably decimates the cell viability and growth ( $p<0.001$ ). However, Zebularine shows cytotoxic effect after 24 hours. At the same time, Zebularine causes significant change on methylation in caspase-3 ( $p<0.001$ ), caspase-8 ( $p<0.01$ )

and caspase-9 ( $p<0.001$ ) genes in SKBR3 cells. These results support that Zebularine can be a new anticancer agent in epigenetic therapies in breast cancer cells.

### E-P17.03

#### Association of HPV infection and cervical dysplasia progression with DNA methylation of genes, connected to cellular stress and oncogenesis

M. Koprinarova<sup>1</sup>, S. Kovachev<sup>2</sup>, I. Dimitrov<sup>3</sup>, D. Nikolova<sup>4</sup>, Z. Hammoudeh<sup>4</sup>, A. Todorova<sup>5</sup>, I. Dimova<sup>4</sup>;

<sup>1</sup>Institute of Molecular biology, Bulgarian Academy of Sciences, Sofia, Bulgaria,

<sup>2</sup>Gynecology Unit, Military Medical Academy, Sofia, Bulgaria, <sup>3</sup>SAGBAL "Dr Shterev", Sofia, Bulgaria, <sup>4</sup>Department of Medical genetics, Medical University Sofia, Sofia, Bulgaria, <sup>5</sup>Department of Chemistry and Biochemistry, Medical University Sofia, Sofia, Bulgaria.

Cervical cancer is tightly connected to HPV infection, but it is not yet elucidated why some of infected cases progress to cancer, but others not. Our aim was to study the role of DNA methylation in this process by analyzing HPV-infected cervical tissues from different PAP groups.

We measured unmethylated and hypermethylated fraction in promoters of 22 genes, connected to cellular stress and toxicity by real-time PCR in three groups: (i) 10 HPV positive PAP I/II cervical samples; (ii) 10 HPV positive PAP III/IV cervical samples; (iii) 10 healthy blood donors.

Overall, there was an increased average hypermethylated fraction (Fhm) for all investigated genes in cervical groups compared to controls. The highly methylated genes were DNAJC15 (Fhm 31% and 32% vs 0.6% in controls), BRCA1 (29% and 13% vs 0.8%), Tp53 (19.5% and 19.2% vs 0.8%), Gpx3 (13% and 16% vs 1.3%), ATM (11% and 15% vs 0.7%), CSTB (11% and 13% vs 0.3%) and Prdx2 (11.7% and 11.6% vs 0.5%). Comparison between high grade and low grade lesions found an increase in Fhm of more than 1.5 times for the genes Msh2 (3 times), Rara (2.1 times), Gadd45G (1.7 times), SCARA3 (1.6 times) and Mlh1 (1.5 times).

We suggest an association between HPV infection and hypermethylation (inactivation) of important tumor-suppressor genes and genes, connected to cellular stress. Dysplasia continues to progress along with further hypermethylation (inactivation) of the mismatch repair genes and genes connected to inflammation response and oxidative stress protection.

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### E-P17.06

#### DNA methylation levels in formalin induced orofacial pain

E. Abzianidze<sup>1,2</sup>, E. Kvaratskhelia<sup>1</sup>, T. Tkemaladze<sup>1</sup>, G. Gurtskaia<sup>2</sup>, N. Tsiklauri<sup>2</sup>, M. Nebieridze<sup>2</sup>, L. Nozadze<sup>2</sup>, M. Tsagareli<sup>2</sup>;

<sup>1</sup>Tbilisi State Medical University, Dept. of Molecular and Medical Genetics, Tbilisi, Georgia, <sup>2</sup>Ivane Beritashvili Centre of Experimental Biomedicine, Dept of Pain and Analgesia, Tbilisi, Georgia.

Introduction: The aim of the present study was to evaluate the changes of a subset of DNA methyltransferases and global DNA methylation level in the anterior cingulate cortex (ACC) and the trigeminal ganglia (TG) neurons in formalin induced orofacial pain.

Materials and Methods: Adult rats were injected with 50  $\mu$ L of 2.5% v/v formalin subcutaneously into upper lip and consequent facial grooming behavior was monitored. All animal studies conformed to the Guidelines of International Association for the study of Pain regarding investigations. The levels of DNMT1, DNMT3a and DNMT3b were measured in nuclear extracts of the ACC and the TG neurons using DNMTs assay kits (Abcam); Genomic DNA was isolated from ACC and TG neurons using DNA isolation universal kit (Zymo Research) and Global DNA methylation was measured using the Methylated DNA Quantification Kit (abcam) in 1,5 hours after formalin injection.

Results: The 5-methylcytosine levels in rat genomic DNA obtained from the TG and the ACC neurons were decreased in the study group compared with the control group. No significant differences in global methylation level between the TG and the ACC were observed. In addition, DNMT3a and DNMT3b were overexpressed in nuclear extracts of the TG and the ACC neurons from rats with formalin induced pain compared with control sample, whereas slight increase in DNMT1 levels was detected in study group.

Conclusions: The results demonstrate that changes in global DNA methylation are correlated with DNMT3a/b levels and these epigenetic mechanisms may be involved in the development and continuation of chronic pain.

**E-P17.08****Intimate Partner Violence and Post Traumatic Stress Disorder: looking for epigenetic markers associated to violence against women**

*S. F. M. Gaudi<sup>1</sup>, A. A. Piccinini<sup>2</sup>, E. Longo<sup>1</sup>, A. Pitudis<sup>1</sup>, A. Kustermann<sup>2</sup>, D. A. Coviello<sup>2</sup>, C. Baldo<sup>4</sup>, P. Cremonesi<sup>4</sup>, S. Polidoro<sup>5</sup>;*

<sup>1</sup>Italian National Institute of Health, Roma, Italy, <sup>2</sup>University of Milan, Milan, Italy, <sup>3</sup>E. O. Galliera, Genoa, Italy, <sup>4</sup>E.O. Galliera, Genoa, Italy, <sup>5</sup>Hugef, Turin, Italy.

**Introduction:** violence against women is a relevant health and social problem. The interactions between genome/epigenome and environmental factors, such as Intimate Partner Violence, represent one of the major challenges in molecular medicine. The major focus of our research project, epi-REVAMP (from the Latin REpellere Vulnra Ad Mulierem et Puerum), is the characterization of the epigenetic profile of women that experienced IPV, and the identification of epigenetic biomarkers that are able to distinguish between women at high and low risk to develop Post Traumatic Stress Disorder.

**Materials and Methods:** we expect to study 600 subjects/year in the SINICA-IDB (Security in Domestic Environment National surveillance Project) surveillance. DNA and RNA samples will be collected in a cohort of 200 women who suffered violence and 400 matched controls of women who did not. The epigenome will be analysed using the array Infinium HumanMethylation450 BeadChip (Illumina,USA). The study protocol has been reviewed and approved by the Ethics Committee of the Italian National Institute of Health.

**Results:** both early detection of post-traumatic distress and the identification of epigenomic factors are the fundamental scope of this research which is focused on the resilience or not resilience to PTSD. This innovative approach represents a shift in the understanding of genetics from a gene-centric view to the phenotypic plasticity of a given genotype producing different phenotypes in response to different environmental conditions, allowing future targeted psychological and pharmacological therapies.

**E-P17.09****Interaction of prenatal maternal stress and a polygenic risk score for transcriptome response on methylation in cord blood: results from the PREDO study**

*D. Czamara<sup>1</sup>, S. Jurato<sup>1</sup>, J. Arloth<sup>1</sup>, J. Lahti<sup>2</sup>, K. Raikkonen<sup>2</sup>, E. Kajantie<sup>3,4,5</sup>, P. Villa<sup>6</sup>, H. Laijuuri<sup>7,8</sup>, E. Hämäläinen<sup>9</sup>, E. Binder<sup>1</sup>,*

<sup>1</sup>Department of Translational Research in Psychiatry, Max-Planck-Institute of Psychiatry, Munich, Germany, <sup>2</sup>Faculty of Behavioural Sciences, Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland, <sup>3</sup>National Institute for Health and Welfare, Helsinki, Finland, <sup>4</sup>Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland, <sup>5</sup>PEDEGO Research Unit, MRC Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland, <sup>6</sup>Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>7</sup>Medical and Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, <sup>8</sup>Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland, <sup>9</sup>HUSLAB and Department of Clinical Chemistry, Helsinki University, Helsinki, Finland.

**Introduction:** It has been shown that prenatal maternal stress can have high impact on the child's development and health. These prenatal environmental conditions can often be tracked down in the child's epigenome, e.g. changing the child's methylation profile.

**Materials and Methods:** The PREDO study is a cohort follow-up study of Finnish pregnant women with and without risk factors for pre-eclampsia. Within these study cord blood samples of 996 children and for a subsample also placenta and mothers' DNA samples have been collected. Different metabolic and psychiatric phenotypes were assessed for the mothers including type 1 diabetes, hypertension, BMI, anxiety and depression rating scales. 996 cord blood samples were genotyped using the Illumina OmniExpress-Exome Array and DNA methylation levels were assessed using the Infinium HumanMethylation450 BeadChip.

We focused on the polygenic risk score derived from Arloth et al. (1) who showed that SNPs that alter the initial transcriptome response to glucocorticoid receptor activation also increase the risk for stress-related psychiatric disorders.

We ran an interaction analysis between this risk score and maternal stress on methylation.

**Results:** analysis is still ongoing at the moment of abstract submission. We aim to identify methylation sites which are modulated by both genetic and environmental factors.

**Conclusion:** These results as well as future analysis on the interaction pattern on whole genome and whole methylome level will give new insights into epigenetic programming.

(1) Arloth et al. Genetic Differences in the Immediate Transcriptome Response to Stress Predict Risk-Related Brain Function and Psychiatric Disorders. *Neuron*. 2015 Jun 3;86(5):1189-202.

**E-P17.10****microRNA-34b, -21 and -133a expression pattern in thyroid carcinoma**

*A. Plesa<sup>1</sup>, I. V. Iancu<sup>1</sup>, A. Botezatu<sup>1</sup>, I. Huica<sup>1</sup>, D. Manda<sup>2</sup>, S. Vladoiu<sup>2</sup>, G. Anton<sup>1</sup>, C. V. Badiu<sup>2</sup>;*

<sup>1</sup>Stefan S Nicolau, Bucharest, Romania, <sup>2</sup>CI Parhon " National Institute of Endocrinology, Bucharest, Romania.

Thyroid cancer is a common type of endocrine malignancy and initiation and progression involves multiple genetic and epigenetic alterations (e.g. microRNA (miRNA) deregulation and aberrant gene promoter methylation). Recent studies have shown a difference in expression of certain miRNAs between normal thyroid tissue and tumors tissue and have highlighted their potential as diagnostic markers. Study aims to examine expression patterns of studied miRNAs in thyroid cancer.

Total RNA was extracted from histological confirmed cancer and non-cancer thyroid tissue from 40 patients (matched thyroid cancer tissue and their adjacent normal tissues as controls) using TriZol. MiRNAs expression levels were estimated in qRT-PCR with RNU43 as reference. Statistical analysis was performed with GraphPad Prism.

miR-34b was found to be down-regulated (<2 fold, P<0.003), the lowest expression was noted in papillary thyroid carcinoma patients (n-fold =-45.69-18.24) compared with papillary thyroid cancer-follicular (n-fold =23.54-2.650) and sclerosing variant (n-fold =-17.66- -5.120). Results indicate a significantly increased miR-21(n-fold=5.27- 16.48) and miR-133 (n-fold =0.40- 56.82) expression in papillary thyroid cancer subjects (P<0.001). Moreover, we observed that miR133a presented the highest expression level in papillary thyroid cancer follicular variant, whereas miR-21 displayed a high expression in papillary thyroid cancer follicular variant, but also in sclerosing variant.

The study presented a characteristic pattern for studied miRNAs gene expression in different thyroid cancer types, raising the possibility to establish specific biomarkers. Nevertheless, further studies are needed to establish the relationship between miRNAs expression and papillary thyroid carcinoma variants.

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**E-P17.11****Association of methylenetetrahydrofolate reductase (MTHFR) gene 677 C > T polymorphism with epigenetic changes in Georgian patients with primary hypothyroidism unspecified**

*T. Kvaratskhelia<sup>1</sup>, E. Kvaratskhelia, K. Kankava, E. Abzianidze; Tbilisi State Medical University, Tbilisi, Georgia.*

**Introduction:** A number of studies have demonstrated that the polymorphism 677C> T in MTHFR gene leads to decreased activity of the enzyme and its association with thyroid disorders. The aim of our study was to investigate the association between MTHFR gene 677 C > T polymorphism and DNMTs levels in patients with primary hypothyroidism.

**Materials and methods:** In this study 16 adult patients with primary hypothyroidism - unspecified and 14 healthy controls (mean age 29 ± 8.5 and 25 ± 9.8 years respectively) were selected. All patients were diagnosed based on serum levels of TSH, FT4, anti-TG and anti-TPO antibodies. Written informed consents were obtained from all study subjects. Genomic DNA was extracted using quick-DNA universal kit (Zymo Research). The 677C> T MTHFR polymorphism was genotyped by PCR- RELP. Levels of DNMT1 and 3a were measured in nuclear extracts of PBMC using DNMTs assay kits (Abcam).

**Results:** The frequency of 677TT genotype was significantly different among the patient and the control group. Genotype frequencies for the patients and controls, respectively, were: 18.75% and 7.1% for 677TT, 37.5% and 28.6% for 677CT, 43.75% and 64.3% for 677CC. In addition, individuals with TT genotype and hypothyroidism showed elevated amount of DNMT3a in nuclear extracts of PBMC compared with controls, while no significant difference in DNMT1 levels was observed.

**Conclusions:** This initial study indicates that the MTHFR 677TT genotype correlates with DNMT3a levels and could contribute to the development of primary hypothyroidism. However, studies with larger samples are required to confirm these findings.

## E-P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

### E-P18.02

#### Clinical and molecular findings in three Moroccan families with distal renal tubular acidosis and deafness: report of a novel mutation of ATP6V1B1 gene

L. Boualla<sup>1,2</sup>, w. jdioui<sup>1,3</sup>, i. ratbi<sup>4</sup>, k. soulami<sup>5</sup>, a. sefiani<sup>6,7</sup>;

<sup>1</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, RABAT, Morocco, <sup>2</sup>Centre de Génomique Humaine, Faculté de Médecine et de Pharmacie, Université Mohammed V, Rabat, Morocco, Rabat, Morocco, <sup>3</sup>Centre de génomique humaine, Faculté de Médecine et de Pharmacie, Université Mohammed V Souissi, Rabat, Morocco, <sup>4</sup>Centre de génomique humaine, Faculté de Médecine et de Pharmacie, Université Mohammed V Souissi, RABAT, Morocco, <sup>5</sup>Cabinet of pediatric Nephrology, Casablanca, Morocco, <sup>6</sup>Centre de génomique humaine, Faculté de Médecine et de Pharmacie, Université Mohammed V Souissi, RABAT, Morocco, <sup>7</sup>Département de Génétique Médicale, Institut National d'Hygiène, Rabat, Morocco, Rabat, Morocco.

**Background:** Primary distal renal tubular acidosis (dRTA) is a rare genetic condition characterized by an impaired acid excretion by the intercalated cells in the renal collecting duct. Recessive forms of this disease are caused by mutations in two major genes: ATP6V1B1 and ATP6V0A4. Causal mutations in ATP6V1B1 gene are classically associated with early sensorineural hearing loss, however cases of tubular acidosis with early deafness have also been described in patients with mutations in the ATP6V0A4 gene.

**Methods:** The phenotype and genotype of three Moroccan consanguineous families with dRTA and deafness were assessed. Molecular analysis was performed by PCR amplification and direct sequencing of exon 12 of ATP6V1B1 gene.

**Results:** A novel c.1169dupC frameshift mutation of ATP6V1B1 gene was identified in one family and the c.1155dupC North African mutation in the two other families.

**Discussion and conclusion** In this report, we propose first line genetic testing based on screening of these two mutations both located in exon 12 of ATP6V1B1 gene in Moroccan patients with recessive form of dRTA associated to precocious hearing loss. Molecular diagnosis of dRTA leads to appropriate treatment and prevention of renal failure in affected individuals and to provide genetic counseling for families at risk.

### E-P18.07

#### The influence of neonatal screening program on the early diagnosis of cystic fibrosis in Moscow Region (Russian Federation)

N. Kashirskaya<sup>1</sup>, V. Sherman<sup>1</sup>, N. Kapranov<sup>1</sup>, E. Kondratyeva<sup>1</sup>, A. Voronkova<sup>1</sup>, V.

Nikonova<sup>1</sup>, S. Krasovskiy<sup>2</sup>, A. Cherniak<sup>2</sup>, E. Amelina<sup>2</sup>, N. Petrova<sup>1</sup>, R. Zinchenko<sup>1</sup>;

<sup>1</sup>Federal State Budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>2</sup>Research Institute of Pulmonology, Moscow, Russian Federation.

National newborn screening (NBS) program for CF was introduced all over Russia in January 2007 (in Moscow - June 2006). Currently, this is the main method of CF diagnosis in the country.

We've analyzed the data of all CF patients from the Moscow region CF Patients' Register that were diagnosed before 18 years of age. The CF patients were divided into two groups according to the different time periods: from 1999 to 2005 - Group I - 83 CF patients (before the introduction of NBS in Moscow) and from 2007 to 2013 - Group II - 228 CF patients (after the introduction of NBS).

The average age of diagnosis in Group I was 4.5 years (30% before 12 months), and in Group II - 1.8 years (74% before 12 months). The average age of CF diagnosis in the positive NBS group was 2.3 months, in the infants with false-negative NBS results - 23 months.

In Group I meconium ileus (MI) was diagnosed in 5 children (6%), and in group II - in 27 children (12%).

We assume there were much more cases of CF under-diagnosis in MI newborns before 2007. MI patients in Group I were diagnosed as having CF at the age of 1.4 years, and in Group II - 1.7 months.

Our findings demonstrate the effectiveness of NBS program. At the same time, underdiagnosed CF before NBS, false-negative results of NBS as well as some problems in the CF care still influence the diagnosis age of CF patients.

### E-P18.08

#### Analysis of frequent CFTR mutations in four ethnic groups from the Republic of Karachay-Cherkessia, Russian Federation

N. V. Petrova<sup>1</sup>, N. J. Kashirskaya<sup>1</sup>, A. K. Makaov<sup>2</sup>, T. A. Vasilyeva<sup>1</sup>, E. E. Timkovskaya<sup>1</sup>, R. A. Zinchenko<sup>1,3</sup>, S. Musaeva<sup>1</sup>, E. K. Ginter<sup>1,4</sup>, S. I. Kutsev<sup>1,3</sup>;

<sup>1</sup>Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>2</sup>Municipal Budgetary Health Care setting „Habetskaya central district hospital”, Habets, Russian Federation, <sup>3</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation, <sup>4</sup>Russian Medical Academy of Postgraduate Education, Moscow, Russian Federation.

Cystic fibrosis (OMIM #219700) is autosomal recessive disorder due to mutation in CFTR gene (OMIM #602421). The spectrum and frequency of CFTR mutations varies considerably in different ethnic groups and geographical regions. The aim: to study the frequency of CFTR mutations in Karachay-Cherkess Republic, the region of compact residence of four small peoples: Karachay, Cherkess, Abaza, Nogai.

**Methods:** The analysis of 12 common in Russia CFTR mutations was performed in 653 healthy unrelated individuals from 7 districts and Cherkess city, representing four ethnic groups, by multiplexPCR and restriction analysis.

**Results:** Two carriers of 1677delTA mutation (0.003) and 6 carriers of W1282X mutation (0.01) in 300 Karachay; two 1677delTA carriers (0.01) and two F508del carriers (0.01) in 103 Cherkess; three 1677delTA carriers (0.012), one W1282X carrier (0.004) and one carrier of F508del (0.004) in 128 Abaza and three W1282X carriers (0.012) in 122 Nogai were revealed.

**Conclusion:** In each of the sample from different ethnic groups, the cumulative frequency of detected CFTR mutations was greater than 0.01, thus the CF incidence should be at least 1 in 10000. The 1677delTA mutation is widespread among CF patients, related to the people of North Caucasus (Chechens, Ingushs) and Transcaucasia (Georgians). We found 1677delTA mutation in Karachay, Abaza and Cherkess that are autochthonous ethnic groups of the North-Caucasian region of Russia, and not discovered for Nogai with a more short history of residence on this territory (since XIV century AD). This work was partially funded by RFBR grants 14-04-00525, 15-04-01859.

### E-P18.09

#### Genome-wide reconstruction of the common ancestral haplotype with splice site mutation c.-23+1G>A (GJB2) in some Siberian and Caucasian populations

A. V. Solov'yev<sup>1,2</sup>, N. A. Barashkov<sup>1,2</sup>, O. L. Posukh<sup>3,4</sup>, M. S. Bady-Khoo<sup>3,5</sup>, F. M. Teryutin<sup>1,2</sup>, V. G. Pshennikova<sup>1,2</sup>, G. P. Romanov<sup>1,2</sup>, N. N. Gotovtsev<sup>1</sup>, I. V. Morozov<sup>4,6</sup>, A. A. Bondar<sup>2</sup>,

A. M. Rafailov<sup>1</sup>, N. N. Sazonov<sup>1</sup>, A. N. Alexeev<sup>7</sup>, L. U. Dzhemileva<sup>8</sup>, E. Metspalu<sup>9</sup>, E. K.

Khusnutdinova<sup>8,10</sup>, M. Metspalu<sup>9</sup>, R. Villem<sup>9</sup>, S. A. Fedorova<sup>1,2</sup>,

<sup>1</sup>M.K. Ammosov North-Eastern Federal University, Yakutsk, Russian Federation, <sup>2</sup>Yakut Scientific Center of Complex Medical Problems, Yakutsk, Russian Federation, <sup>3</sup>Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation, <sup>4</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>5</sup>Tyva Republican Perinatal Center, Kyzyl, Russian Federation, <sup>6</sup>Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation, <sup>7</sup>Institute of Humanitarian Research and Indigenous Peoples of the North, Siberian Branch of the Russian Academy of Sciences, Yakutsk, Russian Federation, <sup>8</sup>Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russian Federation,

<sup>9</sup>Estonian Biocentre, Tartu, Estonia, <sup>10</sup>Bashkir State University, Ufa, Austria.

**Introduction:** The c.-23+1G>A (IVS1+1G>A) splice site mutation is one of the most frequent mutations of GJB2 gene (MIM 121011) in patients with congenital deafness in some populations of Eastern Europe, Caucasus, Middle East, Central and South Asia, Southern and Eastern Siberia. In recent study, we provided evidence that high prevalence of this splice site mutation among Yakut population isolate in Eastern Siberia is due to common founder effect [Barashkov et al., 2011]. The aim of this study is to investigate the origin of the c.-23+1G>A mutation found in some Siberian and Caucasian (Russian) populations.

**Materials and methods:** DNA samples of four deaf patients with c.-23+1G>A mutation (GJB2) in homozygous state representing several ethnic Eurasian populations (Yakut, Russian, Tuvian and Evenk) were genotyped with Illumina 730K SNP array according to manufacturers' specifications (Estonian Biocentre, Tartu).

**Results:** The presence of extended homozygous region (from ~1.4 Mb in Russian to ~7.6 Mb in Tuvian) was shown for all studied patients. Moreover, we have found identical, common for all studied individuals, homozygosity track (~325 kb) flanking the region of c.-23+1G>A mutation.

**Conclusions:** Thus, the results of the genome-wide analysis support the hypothesis of a common origin of the c.-23+1G>A splice site mutation (GJB2) in certain populations of Eurasia. The study was supported by the RFBR grant #16-34-00234, the governmental contract #6.656.2014/K, and the grant #0324-2015-0031 from the Siberian Branch of Russian Academy of Sciences.

**E-P18.11****STR polymorphism of CFTR gene in nine populations of Russia**

E. E. Tymkovskaya<sup>1</sup>, N. V. Petrova<sup>2</sup>, A. K. Makaov<sup>3</sup>, T. A. Vasilyeva<sup>2</sup>, S. M. Musaeva<sup>2</sup>, R. A. Zinchenko<sup>2,4</sup>;

<sup>1</sup>Research Center for Medical Genetics, Moscow, Russian Federation, <sup>2</sup>Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>3</sup>Municipal Budgetary Health Care setting „Habetskaya central district hospital”, Habets, Russian Federation, <sup>4</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Molecular genetic analysis of four STR polymorphisms in CFTR gene IVS1CA-IVS6aGATT-IVS8CA-IVS17bCA was performed in nine populations from Russian Federation. Four Northern-Caucasus populations from the Karachay-Cherkess Republic (Karachai, Cherkess, Abaza, Nogai) and five populations from Volgo-Urals region (Mary, Udmurt, Bashkir, Tatar, Chuvashes) were included in the survey. Cherkess and Abaza are North Caucasian ethnic groups closely related to Abkhaz, Nogai and Karachai are Turkic people, Nogai descendants of various Mongolic and Turkic tribes, Karachai from the Kipchaks.

Mary and the Udmurt are Finno-Ugric peoples, Chuvashes descends from Proto Turkic people, Suars and Bulgars, Tatars - from the Volga Bulgars and Turkic tribes, Bashkir - from the different tribes of Finno-Ugric and Turkic tribes. The volume of each sample was 55-60 unrelated healthy individuals. Allele and genotype frequencies as well as indices of intrapopulational differentiation ( $F_{ST}$ ) were calculated.

The intrapopulational differentiation for all studied populations is ( $F_{ST}$ ) 0,0271. The intrapopulational differentiation among populations from Volgo-Urals region was considerably higher than that of ethnic groups from the North Caucasus (0,0072 vs 0,0179).

Analysis of genetic distances prompted us to conclude that Mary, Udmurt, and Chuvashes genetically closer to each other than to Tatar and Bashkir. Cherkess and the Abaza are genetically closer to each other than to Karachai and Nogai.

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**E-P18.12****Mitochondrial haplogroup H diversity in Russians based on complete mitogenome analysis**

B. A. Malyarchuk, A. Litvinov, M. Derenko;

Institute of Biological Problems of the North, Magadan, Russian Federation.

Haplogroup H comprises about 50% of mitochondrial DNA (mtDNA) lineages in European populations, including Russians. This haplogroup has been difficult to subdivide based on the hypervariable segments I and II (HVS I and II) variation alone. In Russians, a number of major clades within haplogroup H were revealed on the basis of high-resolution mtDNA analysis (i.e. HVS I and II sequencing combined with analysis of some subhaplogroup-specific coding region sites) - clades H1a, H1b, H2, H4, H5, H6, H7 and H11. However, complete mtDNA analysis at higher levels of phylogenetic resolution allows revealing more than 100 distinct clades within haplogroup H. Therefore, to resolve the phylogeny of this haplogroup in Russians, we sequenced 118 complete H-mitogenomes of Russian individuals from northwestern (Pskov and Velikij Novgorod) and southwestern (Belgorod, Orel and Tula) regions of European part of the Russian Federation. Phylogenetic analysis has shown that the largest subclades in Russians are H1 (about 30% of haplogroup H pool, with lineages H1\*, H1a, H1b, H1c, H1n, H1aj), H5 (about 16%, with lineages H5a, H5b, H5e, H5f, H5u), and H11 (about 10%, with lineages H11a1 and H11a2). Subclades H6, H2, H3 and H13 each comprise about 5% of haplogroup H mitogenomes, while a large number of rare mtDNA lineages (H4, H7, H10e, H13, H14a, H24, H27, H28a, H32, H35, H36a, H41a, H44b, H56a1, H79, H81 and H89) contribute substantially into haplogroup H diversity. This study was supported by Russian Foundation for Basic Research (grant 14-04-00131).

**E-P18.13****The first case of monilethrix DNA diagnosis in Russia**

E. V. Degtereva<sup>1</sup>, S. S. Amelina<sup>1</sup>, N. V. Vetrova<sup>2</sup>, N. V. Petrova<sup>3</sup>, T. A. Vasilyeva<sup>3</sup>, S. I. Kutsev<sup>3</sup>, R. A. Zinchenko<sup>2</sup>;

<sup>1</sup>Rostov State Medical University, Rostov-on-Don, Russian Federation, <sup>2</sup>Academy of Biology and Biotechnology of Southern Federal University, Rostov-on-Don, Russian Federation, <sup>3</sup>Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: Monilethrix (MIM #158000) is a rare genodermatosis characterized by a hair shaft dysplasia resulting in hypotrichosis. The prevalence and incidence are not known. The three main genes are associated with monilethrix: KRT81, KRT83 and KRT86, coding the type II hair keratins Hb1, Hb3 and Hb6, and are responsible for the autosomal dominant form of the

disease.

Materials and methods: Epidemiological, PCR, sequencing.

Results: The population of twelve districts was examined to determine prevalence of hereditary disorders in Rostov Region. We examined 497460 people. Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Research Centre for Medical Genetics.

The study identified 5 patients from 1 family of 3 generations. All patients had the typical symptoms of the disease at an early age. Clinical examination: the hair on the scalp rare, dry, brittle, their length does not exceed 1.5-2 cm, 2 patients in the pathological process involved eyebrows and eyelashes. Direct sequencing revealed a mutation E402K in exon 4 in the gene KRT86 (HB6) in the heterozygous condition.

Conclusions: The prevalence rate monilethrix was 1.01 per 100 000 population (1:99492). The results of the study can be used for optimizing Medical Genetic Counselling in monilethrix families in the Rostov region.

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**E-P18.17****Neurofibromatosis in Rostov Region**

N. Vetrova<sup>1</sup>, S. Amelina<sup>2</sup>, T. Ponomareva<sup>2</sup>, M. Amelina<sup>1</sup>, S. Kutsev<sup>3</sup>, R. Zinchenko<sup>3</sup>;

<sup>1</sup>Academy of Biology and Biotechnology of Southern Federal University, Rostov-on-Don, Russian Federation, <sup>2</sup>Rostov State Medical University, Rostov-on-Don, Russian Federation, <sup>3</sup>Research Centre for Medical Genetics, Moscow, Russian Federation.

Neurofibromatosis is the most frequent inherited disorder associated with increased susceptibility to the development of benign and malignant tumors. Penetrance is 100% but the clinical features of this disorder are highly variable, even within the same family.

The population of twelve districts was examined to determine prevalence of hereditary disorders in Rostov Region. We examined 497460 patients. Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Medical Centre in Moscow.

According to the research results neurofibromatosis is one of the most frequent autosomal dominant hereditary diseases in the population of the Rostov region. The study identified 54 patients from 28 families with typical clinical manifestations of neurofibromatosis type 1. The prevalence rate was 10.9 per 100 000 population (1:9212). Most families have a burdened family history (26 from 28). The tumor is diagnosed in 3 patients, in two cases there are central nervous system tumors, and one patient has benign tumor of the thorax.

Independent inheritance of two diseases neurofibromatosis and retinitis pigmentosa was determined in one family. Mother (who died from central nervous system tumor) had both diseases. There are four siblings from different marriages of the mother. One son has not inherited any of the disease, two daughters inherited both diseases, and the youngest daughter inherited only the retinitis pigmentosa.

The results of genetic and epidemiological studies enabled to determine the prevalence of monogenic hereditary diseases will help to improve the medico-genetic service.

**E-P18.19****Association analysis of five genes polymorphisms in two cohorts of European athletes**

V. Ginevičienė<sup>1</sup>, A. Jakaitienė<sup>1</sup>, S. Drozdovska<sup>2</sup>, L. Tubelis<sup>3</sup>, V. Kučinskas<sup>4</sup>, A. Utkus<sup>1</sup>;

<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>National University of Physical Education and Sports of Ukraine, Department of Sport Biology, Kiev, Ukraine, <sup>3</sup>Lithuanian University of Educational Sciences, Vilnius, Lithuania.

Introduction: The aim of this case-control study was to determine the association of the *ACE* (rs4646994 I/D), *ACTN3* (rs1815739 R/X), *HIF1A* (rs11549465 Pro582Ser), *PPARA* (rs4253778 G/C) and *PPARG* (rs1801282 Pro12Ala) polymorphisms, with athletic status in European Caucasians cohort consisting of Lithuanian and Ukrainian. Selected polymorphisms were previously reported as associated with athlete status in different populations.

Materials and Methods: A total of 499 elite athletes (250 Lithuanian, 249 Ukrainian) and 584 non-athlete controls (healthy unrelated 266 Lithuanian, 318 Ukrainian citizens) were genotyped for mentioned polymorphisms using PCR and/or restriction enzyme digestion. The athletes were stratified into endurance-oriented, power-oriented and group of athletes with mixed endurance/power activity.

Results: The analysis of polymorphisms, except *ACE* (I/D) and *ACTN3* (R577X), demonstrated no significant differences between populations. The *ACE* I allele frequency was higher in Lithuanian power-oriented ath-

letes compared to controls (51% vs. 42.6%; P=0.02). The frequency of the *PPARA* minor C allele was significantly lower in Ukrainian than Lithuanian endurance athletes (17.2% vs. 25%; P=0.031). The proportion of the *PPARG* Ala allele, observed in Ukrainian power-oriented athletes, was larger than in endurance-oriented athletes (24.7% vs. 13.5%; P=0.008). Ukrainian power-oriented athletes exhibited an increased frequency of the *HIF1A* rare Ser allele (16.1% vs. 9.4%; P=0.034) compared to controls.

Conclusions: We conclude that the *ACE* I allele determine power status for Lithuanian athletes. Likewise, *HIF1A* Ser and *PPARG* Ala alleles associated with power athlete status in Ukrainians. We confirm that variants which have a significant association with physical performance in studies of one population may not have the same association in another.

#### E-P18.21

#### Polymorphism of nine nuclear genome DNA loci in Abaza and Nogai

A. K. Makaov<sup>1</sup>, N. V. Petrova<sup>2</sup>, E. E. Timkovskaya<sup>2</sup>, T. A. Vasilyeva<sup>2</sup>, S. M. Musaeva<sup>2</sup>, E. K. Ginter<sup>2</sup>, G. I. El'chinova<sup>2</sup>, S. I. Kutsev<sup>2,3</sup>, R. A. Zinchenko<sup>2,3</sup>;

<sup>1</sup>Municipal Budgetary Health Care setting, Habez, Russian Federation, <sup>2</sup>Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, <sup>3</sup>Pirogov Russian National Research Medical University, Moscow, Russian Federation.

The Karachay-Cherkess Republic, Russian Federation, is a region of compact residence of four different ethnic groups: the Karachai, Cherkess, Abaza, Nogai. The aim was to carry out the population genetic survey of two indigenous populations of the Karachay-Cherkessia Republic: Abaza and Nogai. The Abaza are an ethnic group of the Caucasus, closely related to the Abkhaz and Circassian (Adyge) people. The Nogai, a Turkic ethnic group, are thought to be descendants of various Mongolic and Turkic tribes, who formed the Nogai Horde.

Methods: DNA samples of healthy unrelated individuals (110 Abaza and 122 Nogai) were examined at nine polymorphic DNA loci of nuclear genome: diallele CCR5 (del32), ACE (del/ins), D7S23 (KM19), NOS3 (VNTR), and polyallelic TH01 (STR), FABP2 (STR), CFTR (IVS6aGATT), PAH (VNTR), DAT1 (VNTR) by PCR.

Results: Allele and genotype frequency distributions were obtained for both ethnic groups. Analysis of allele's frequency of autosomal DNA markers revealed genetic differentiation between populations of Abaza and Nogai. The highest levels of genetic diversity in diallele system were established at locus ACE (del/ins), Hobs=0.5963 in Abaza and Hobs=0.5333 in Nogai, in polyallelic system - at locus TH01 (STR), Hobs=0.7773 in Abaza and Hobs=0.7934 in Nogai. The index of mean heterozygosity was higher in Nogai than in Abaza (0.4784 and 0.4555, correspondingly). The index of mean intrapopulation differentiation (FST) was 0.0040. The highest level of intrapopulation differentiation was revealed at locus KM19 (FST=0.0122), the lowest one - at locus TH01 (FST=0.0014).

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#### E-P19 Genetic counselling/Education/public services

#### E-P19.02

#### The novel probably causal substitution in the patient with FSGS

M. Safarikova<sup>1,2</sup>, J. Reiterova<sup>2</sup>, J. Stekrova<sup>1</sup>, V. Horinova<sup>3</sup>, M. Kohoutova<sup>1</sup>, V. Tesar<sup>2</sup>, S. Pospisilova<sup>4</sup>;

<sup>1</sup>Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic, <sup>2</sup>Department of Nephrology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic, <sup>3</sup>Genetic Ambulance Jihlava, Jihlava, Czech Republic, <sup>4</sup>General University Hospital in Prague, Prague, Czech Republic.

Focal segmental glomerulosclerosis (FSGS) is common primary glomerulopathy that often progresses to the end stage renal disease (ESRD). Several genes, such as ACTN4, CD2AP, INF2, NPHS2 and TRPC6, have been identified as a cause of autosomal dominant focal segmental glomerulosclerosis. Alpha-actinin-4, encoded ACTN4 gene, is a member of the spectrin gene superfamily that crosslinks F-actin filaments. It is nonmuscle isoform expressed in many human tissues. The structure is head-to-tail antiparallel homodimer with three main parts. N-terminal domain is composed of two calponin homologous domains with actin binding sites followed by four spectrin repeats. C-terminal domain consists of two EF-hand domains that can bind Ca<sup>2+</sup> ions.

Our patient was thirty-two years old man from Italy, now living in the Czech Republic, with positive family history (mother) suffering from focal segmental glomerulosclerosis who was after renal transplantation. We screened for

mutations in genes ACTN4, INF2, NPHS2 and TRP6 using Sanger sequencing. We identified a novel substitution Leu169Pro in the ACTN4 gene that was in the heterozygous state. According to prediction programs PolyPhen-2 and Mutational Taster this substitution has got the high probability to be causal. We also screened for the new substitution in healthy close relatives (father and brothers) but they are carriers of the wild type sequence. The DNA of the mother who also suffered from FSGS could not be analysed because she is unfortunately dead. In these days we search for the novel substitution in the group of healthy controls using restriction analysis.

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#### E-P19.04

#### Genetic counselling for MELAS during pregnancy - a case report

Y. Goto<sup>1,2</sup>, C. Noji<sup>1</sup>, K. Takahashi<sup>2</sup>, Y. Ohnuki<sup>2</sup>, A. Kondo<sup>3</sup>, T. Muramatsu<sup>1</sup>, S. Izumi<sup>2</sup>;

<sup>1</sup>Dept. of Obstetrics and Gynecology, Tokai University Hachioji Hospital, Tokyo, Japan,

<sup>2</sup>Dept. of Genetics, Tokai University Hospital, Kanagawa, Japan, <sup>3</sup>Dept. of Obstetrics and Gynecology, Shikoku Medical Center for Children and Adults, Kagawa, Japan.

[Introduction] Mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like syndrome (MELAS) is a maternal inheritance and diagnosed by mutation of mitochondrial gene, 3243AG. Symptom varies epilepsy, head ache, vomiting, hard of hearing, mental disorder, short stature, stroke and so on. Affected pregnant women should be monitored for diabetes mellitus and respiratory insufficiency, which may require therapeutic interventions. We experienced a case of pregnancy with MELAS.

[Case] 36 y/o para 0 gravida 0 woman, who is 144cm in height and has a hard of hearing from 30 y/o, conceived by IVF-ET after 9 years infertility and came to our hospital at 9 weeks. At 21 weeks, she suddenly complained headache and dysarthria. Left temporal lobe edema and cerebellum atrophy were observed in MRI. The next day, she had strong uterine contraction which led a miscarriage. One month later, she visited our genetic clinic for asking about some influences to next pregnancy.

[Discussion] MELAS is inheritable from mother by 100%. However permeability is various. The mother of this case has only hard of hearing. The women with genetic disease sometimes confront with complications during pregnancy due to physiological changes. Possibility of prenatal diagnosis is important information, however prenatal diagnosis of MELAS is impossible. [Conclusion] Parental genetic counseling is important for women with any genetic disease before pregnancy. As infertility is not rare condition for MELAS, medical staffs at fertility clinics also should pay attention to their conditions. The patient need sufficient information about their possible problems related pregnancy before the treatment for sterility.

#### E-P19.05

#### Analysis of maternal polymorphisms of RFC1 gene and risk of Down syndrome offspring

A. Pazarbasi, L. Ozpak, N. Keser, S. Kocaturk-Sel, G. Comertpay, F. T. Ozgunen; University of Cukurova, Faculty of Medicine, Adana, Turkey.

BACKGROUND: Down syndrome (DS) is the most common trisomy in live born with a prevalence of 1 in 1000 to 1 in 1100. Enzymes involved in one carbon and transsulfuration (1C/TS) metabolism in mothers and affected individuals with Down syndrome in altering the likelihood of birth of a child with Down syndrome. An important gene located on chromosome 21 encodes the reduced folate carrier1 (RFC1), widely considered to be quantitatively one of the most significant proteins involved in uptake of reduced folates from the diet and in cellular internalisation of reduced folates. We have looked for an association of polymorphisms in the RFC gene and the risk of birth of a child with Down syndrome

MATERIALS AND METHODS: In our study, sixty-one DS mothers and fifty mothers who had no children with DS from Cukurova region of Turkey were evaluated. Genomic DNA was isolated from whole peripheral blood collected on EDTA, using salting out method. The CBS genotypes were studied by PCR-amplified products.

RESULTS: The results show that the frequencies of RFC1 alleles, as well as the frequencies of RFC1 A80G genotypes (AA, GA, GG) do not correlate with DS pregnancies, demonstrating no difference between the case and control groups.

CONCLUSIONS: In the present study, we did not find any statistically significant association between RFC-1 polymorphic genotype and history of DS pregnancies.

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## E-P19.06

**Unusual structural abnormalities of small acrocentric chromosomes-difficulties of genetic counseling**

*S. Popa<sup>1</sup>, R. Popescu<sup>1</sup>, E. Braha<sup>1</sup>, M. Gramescu<sup>1</sup>, E. Graur<sup>1</sup>, V. Martiniciu<sup>2</sup>, C. Rusu<sup>1</sup>, E. V. Gorduza<sup>1</sup>;*

<sup>1</sup>*Medical genetics department, University of Medicine and Pharmacy "Gr. T. Popa", Iasi, Romania, <sup>2</sup>Medical genetics department, "Cuza-Voda" Obstetrics and Gynecology Hospital, Iasi, Romania.*

Small acrocentric chromosomes are frequently involved in genetic disorders. The majority are de novo abnormalities, but some can be inherited. In last 10 years in Cytogenetic Laboratory of Iasi, were performed 2614 karyotypes with 649 (24.8%) abnormalities in G group chromosomes: 534 (82%) cases of 21 homogeneous trisomy, 38 (6%) cases of mosaic 21 trisomy, 31 (5%) cases of Robertsonian translocations, 34 (5.3%) cases of microdeletions 22q11.2 and 11 (1.7%) cases of unusual structural abnormalities. All cases were investigated by: G banding karyotype, C banding karyotype, NOR banding karyotype, FISH with different fluorescent probes. 9 of unusual unbalanced structural abnormalities were discovered in plurimaleformative children: 46,XX,add(21)(q22.3); 46,XX,dup(21)(q22.3;q11.2); 46,XY,+9,del(9;22)(q12;p11.2); 46,XY,ins(9;21)(p22;q22→qter); 47,XY,t(1;2)(p32→pter)(q37→qter),-3,-21,+der(3)rcp(3;21)(p11.1;q22.2),+der(21)rcp(3;21)(p11.1;q22.2),+21; 46,XY,der(13)(13qter→13q12::21q22.1→21pter); 47,XY,ins(21;18)(p11.2;p11.3→pter),+21; 45,XX,der(5)t(5;22)(5p15.1;22q11.1),-22/46,XX,der(5)t(5;22)(5p15.1;22q11.1); 45,XX,der(5),t(5;21)(5p15;21q)-21 and two with balanced chromosomal abnormality were discovered in couples with infertility (46,XY,ins(21;16)(q22.1;p12.1-p13.13); 45,XX,der(18)t(18;22)(q23;q12),-22). In 6 cases, we identified an inherited balanced abnormality from one parent. All cases needed genetic counseling, in order to identify the risk of next pregnancies. Our retrospective study was focused to identify cases with atypical cytogenetic rearrangements (i.e. some rare variants of chromosomal abnormalities). An important outcome of this study is directly related to couple counseling. Each case was compared with similar cases from literature data. This study was partial supported by founding of PN-II-PT-PCCA-2013-4-133 Program of UEFISCDI (National Romanian organism of research).

## E-P19.07

**Incomplete Timothy syndrome secondary to a mosaic mutation of the CACNA1C gene diagnosed using next-generation sequencing**

*A. Baurand<sup>1</sup>, S. Falcon Eicher<sup>2</sup>, G. Laurent<sup>3</sup>, E. Villain<sup>4</sup>, C. Bonnet<sup>2</sup>, C. Thauvin<sup>1</sup>, C. Jacquot<sup>1</sup>, V. Probst<sup>5</sup>, J. Gourraud<sup>5</sup>, S. Schmitt<sup>6</sup>, S. Bézieau<sup>6</sup>, M. Giraud<sup>6</sup>, S. Dumont<sup>6</sup>, A. Burquet<sup>7</sup>, F. Kyndt<sup>8,9</sup>, L. Olivier Faivre<sup>1</sup>;*

<sup>1</sup>*Service de Génétique CHU DIJON, DIJON, France, <sup>2</sup>Cardiologie Pédiatrique et Congénitale Adulte CHU DIJON, DIJON, France, <sup>3</sup>Centre de compétence des Troubles du Rythme Cardiaque Héréditaires CHU DIJON, DIJON, France, <sup>4</sup>Cardiologie pédiatrique, Hôpital Necker-Enfants Malades, PARIS, France, <sup>5</sup>L'institut du thorax, INSERM U1087, CHU Nantes, NANTES, France, <sup>6</sup>Service de génétique médicale, CHU Nantes, NANTES, France, <sup>7</sup>Réanimation Pédiatrique, CHU Dijon, DIJON, France, <sup>8</sup>L'institut du thorax, INSERM U1087, CHU Nantes, Nantes, France.*

Autosomal dominant genetic diseases can occur de novo and in the form of somatic mosaicism, which can give rise to a less severe phenotype, and make diagnosis more difficult given the sensitivity limits of the methods used. We report the case of a 6-year-old female patient with a history of surgery for syndactyly of the hands and feet, who was admitted to a pediatric intensive care unit following cardiac arrest. The ECG showed a long QT interval that on occasions reached 500 ms and a wide T wave. Despite the absence of facial dysmorphism and the presence of normal psychomotor development, a diagnosis of Timothy syndrome was made given the association of Syndactyly and the electrocardiogram features. Sanger sequencing of the CACNA1C gene, followed by sequencing of the genes KCNQ1, KCNH2, KCNE1, KCNE2, were negative. The subsequent analysis of a panel of genes responsible for hereditary cardiac rhythm disorders using Haloplex technology revealed a recurrent mosaic p.Gly406Arg missense mutation of the CACNA1C gene in 18% of the cells. This mosaicism can explain the negative Sanger analysis and the moderate phenotype in this patient. Given the other cases in the literature, mosaic mutations in Timothy syndrome appear more common than previously thought. This observation shows the interest of using next-generation sequencing to identify mosaic mutations compatible with a clinical picture but not detected using classical technologies for genetic counseling and the management of patients and their families.

## E-P19.08

**Genetics Made Easy**

*M. Solé Pujo<sup>1</sup>, C. Bach Vallmajor<sup>2</sup>, J. Sabrià Rius<sup>2</sup>, J. Antich Femenias<sup>1</sup>;*

<sup>1</sup>*Centre de Genètica Mèdica, Barcelona, Spain, <sup>2</sup>Hospital Universitari Doctor Josep Trueta, Girona, Spain.*

It is a non-profit web site on human genetics, the objective of which is to

bring the scientific community closer to the general community in order to disseminate the advances and knowledge that arise in this field and how the general population can benefit from these developments. The language used in the website is simple and the terminology easy to comprehend, with clear and straight forward explanations and user-friendly software.

This web site is not intended as personalized medical care, but as a complement to it. It can be a very useful tool for the clinician and other healthcare professionals, irrespectively of their area of expertise, as genetic disorders are known across all medical specialties.

**Index:** The origin of life • Cell specialization • Chromosomes • What is heredity and how do we acquire it? • Types of inheritance • Why do disorders develop? • What happens when our recipes combine with our partner's recipes? • Origin of hereditary disorders • How can we use this vast knowledge? • Assisted Reproduction Techniques • Prenatal Diagnosis Techniques • Where are we now and where are we headed • Human Genome Project • Gene therapy • Cloning and stem cells • Genetics and cancer • Farewell.

Updated 2016. <http://www.geneticsmadeeasy.com>

## E-P20 Psychological/Ethical/legal issues

## E-P20.01

**Association of SLC6A4 genotypes with antisocial behavior in response to childhood environment: a study in young adults supporting the hypothesis that 5-HTTLPR is a genetic marker of differential susceptibility**

*L. Manco<sup>1,2</sup>, A. Soares<sup>2</sup>, S. N. Wasterlain<sup>1,2</sup>;*

<sup>1</sup>*Department of Life Sciences, University of Coimbra, Coimbra, Portugal, <sup>2</sup>Research Centre for Anthropology and Health (CIAS), University of Coimbra, Portugal.*

**Introduction:** The SLC6A4 gene encodes the serotonin transporter (5-HT) involved in the reuptake of serotonin at brain synapses. Several studies described an association between the functional repeat length polymorphism 5-HTTLPR, with two common repeat elements (14R; S-allele and 16R; L-allele), and impulsivity, aggression, and conduct disorders. This study aims to replicate the association between 5-HTTLPR and antisocial behavior (ASB) in response to childhood environment (CE) in a general population sample of young adults.

**Materials and Methods:** A sample of 205 (103 males; 102 females) Portuguese healthy individuals, aged 18-37 years, was enrolled in the study. Both ASB and CE were assessed by self-reports using questionnaires. Genotyping was performed for polymorphisms 5-HTTLPR and rs25531 according to published protocols.

**Results:** Allele frequencies (5-HTTLPR L:0.56, S:0.44; rs25531 A:0.95, G:0.05) were similar to those previously reported for European populations and genotype distributions were in Hardy-Weinberg equilibrium (P=0.866 and P=0.431, respectively). When considering individuals exposed to adverse CE a grading tendency towards ASB was observed in carriers of S-alleles. Linear regression analysis yielded near significant association (P=0.05) between the S-allele and ASB in males. By contrast, in the group not exposed to adverse CE, individuals homozygous for the L-allele were more prone to commit ASB. The L-allele was found significantly associated (P=0.01) with ASB when testing all subjects.

**Conclusions:** The findings showing that the S-allele carriers are more vulnerable to childhood negative environments for ASB outcomes and profit more from positive environmental conditions support the hypothesis that 5-HTTLPR is a genetic marker of differential susceptibility.

## E-P20.02

**Genetic fitness of deaf people in the Sakha Republic (Eastern Siberia, Russia)**

*G. P. Romanov<sup>1,2</sup>, N. A. Barashkov<sup>1,2</sup>, F. M. Teryutin<sup>1,3</sup>, L. A. Klarov<sup>3,4</sup>, V. G. Pshennikova<sup>1,2</sup>, L. A. Nikolaeva<sup>4</sup>, O. N. Garyukhina<sup>4</sup>, E. D. Skryabina<sup>4</sup>, A. V. Solovyev<sup>1,2</sup>, N. N. Gotovtsev<sup>2</sup>, N. N. Sazonov<sup>1</sup>, I. V. Morozov<sup>5,6</sup>, A. A. Bondar<sup>5</sup>, A. N. Alekseev<sup>7</sup>, L. U. Dzhemileva<sup>8,9</sup>, E. K. Khusnutdinova<sup>8,10</sup>, O. L. Posukh<sup>6,11</sup>, S. A. Fedorova<sup>1,2</sup>;*

<sup>1</sup>*North-Eastern Federal University, Yakutsk, Russian Federation, <sup>2</sup>Yakut Scientific Center of Complex Medical Problems, Yakutsk, Russian Federation, <sup>3</sup>Republican Hospital # 2 – Center of Emergency Medicine, Ministry of Public Health of the Sakha Republic, Yakutsk, Russian Federation, <sup>4</sup>Yakutsk Branch of All-Russian Society of the Deaf, Yakutsk, Russian Federation, <sup>5</sup>Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation, <sup>6</sup>Novosibirsk State University, Novosibirsk, Russian Federation, <sup>7</sup>Institute for Humanities Research and Indigenous Studies of the North, Siberian Branch of Russian Academy of Sciences, Yakutsk, Russian Federation, <sup>8</sup>Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russian Federation, <sup>9</sup>Bashkir State Medical University, Ufa, Russian Federation, <sup>10</sup>Bashkir State University, Ufa, Russian Federation, <sup>11</sup>Federal Research Center, Institute of Cytology and Genetics, Novosibirsk, Russian Federation.*

Introduction of a sign language in schools for deaf people led to growth of their genetic fitness, which has doubled the GJB2 gene associated deafness

in the USA over the past 200 years [Arnos et al, 2008]. High prevalence of the GJB2-deafness [Barashkov et al, 2011] and relatively recent (~60 years ago) introduction of sign language among deaf people were recorded for indigenous Yakut population (Eastern Siberia, Russia). We have performed study of fertility of deaf people living in Eastern Siberia in comparison with their hearing siblings. Fertility was determined as the average number of children born to examined groups, genetic fitness of deaf people was calculated as the ratio of the overall fertility of deaf individuals to their hearing siblings [Blanton et al, 2010]. Data on fertility of 83 deaf people (females-53, males-30) and 185 hearing siblings (females-88, males-97) aged 35-69 years were collected: deaf individuals have in total 143 children, whereas hearing siblings-422 children. Fertility of deaf people was 1.72 vs 2.28 of their hearing siblings, genetic fitness for deaf individuals was 0.75. There was no difference between genders. Our results are comparable with fitness of deaf women in Sweden - 0.76 [Carlsson et al, 2005], lower than in USA-0.88 [Blanton et al, 2010], and higher than in Mongolia-0.62 [Tekin et al, 2010]. Thus, genetic fitness of deaf people in Eastern Siberia is slightly reduced compared to their hearing siblings. The study was supported by the RFBR grants #15-04-04860\_a, #16-34-00564\_mol\_a, and the #6.656.2014/K MES\_RF project.

## E-P20.05

### Policy proposal addressing blood-borne infections at medical schools in Angola

**M. M. Chimpolo, M. M. Chimpolo;**

Medical School, Universidade A Neto, Luanda, Angola.

Blood borne infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are major Public Health issues throughout the world and Angola is no exception. For instance, more than 166 000 of Angolans are infected with HIV. The transmission of these blood-borne infections in health care settings and particularly amongst medical students brings considerable ethical and legal concerns. In Angola, there are currently no public policies that provides guidance on certain procedures and circumstances where a student carries a blood-borne virus. There is also no requirements for screening or immunisation programs at medical schools.

This paper aims at establishing the prevalence of infected students and providing a reasonable policy proposal that addresses important questions of public health, which may affect both students and patients and prevent the spreading of infections.

The prevalence of HIV, HBV and HCV in 160 first year Angolan medical students was analysed by serological and molecular testing. The review of literature on laws and bioethics regarding these infections were used to gather appropriate data.

The resulting work defines a set of Medical faculty requirements proposals for screening, immunisation, specialized medical assistance and compliance by students in relation to blood-borne virus infections. This policy proposal also includes professional standards and effective policies related to medical management of infections and place responsibilities on those who act negligently and fail to protect their patients

## EMPGAG SPOKEN PRESENTATIONS

### EE1 Educational Session: DTC genetic testing revisited: empowering patients -caring for consumers?

#### EE1.1

##### Shifting roles and relationships: the impact of direct-to-consumer genetic testing on healthcare delivery

**E. Gordon;**

23andMe, Inc., Mountain View, CA, United States.

Historically, healthcare and health information were delivered through trained medical professionals, who also recommended actions for patients to follow (e.g. undergo further tests, take a prescribed medication, etc.). Patients had some autonomy of choice, but in today's digital age, patients have become increasingly empowered to take charge of their health. One example is direct-to-consumer genetic testing (DTCGT). It has a clear public demand, but it has received a decidedly mixed reaction in the medical community.

Despite significant concerns from medical professionals and researchers, research on DTCGT has not supported the misperceptions and other concerns. As a genetic counselor working in the DTCGT industry, I support the public's right to access personal genetic information -- when it is delivered in a responsible manner. Key components and principles of any DTCGT company should include respect for consumer rights and privacy; reports written at an appropriate comprehension level; educational content to support consumers; valid test methods; and compliance with the local regulatory framework. With these in place, research supports consumers ability to comprehend their personal genetic information without evidence of psychological harm. However, the DTCGT industry is well-served to include medical professionals in its ranks. Although supporting consumers of DTCGT is very different from direct patient care, having the clinical experience to create needed resources, identify and correct for potential misperceptions among patients and create key information needed by medical professionals to effectively utilize genetic test information in patient care ultimately lead to better services. Finally, data suggests that physicians are ill-prepared to deal with DTCGT results presented by their patients and that their lack of preparedness may have a negative impact on the patient-physician relationship. DTCGT companies are working to engage and educate medical professionals; an evidence-based assessment and greater acceptance by the medical professional community is also needed.

#### EE1.2

##### The "activated patient": A fresh look at empowerment

**B. Prainsack;**

Department of Social Science, Health & Medicine, King's College London, London, United Kingdom.

Patient empowerment has become a buzzword. It is used to support a great variety of practices ranging from shared decision making in the clinic to self experimentation of 'citizen scientists'. In the domain of DTC genetics, the argument of patient empowerment has been used to support the idea that patients should have direct access to genetic risk information, without the involvement of health professionals. The empowerment rhetoric allows the labeling of those who oppose such ideas as paternalistic empowerment skeptics. This paper seeks to make the of the cultural and political meanings of empowerment more explicit. It suggests that these meanings are considered explicitly when analysing the societal and ethical aspects of DTC genetics. Drawing upon the work of Luca Chiappero and others, I distinguish between three traditions of meaning of empowerment: (1) Empowerment as an expression of individual autonomy and choice (*individualistic empowerment*); (2) empowerment as liberation from paternalism and oppression (*emancipatory empowerment*), and (3) empowerment in the service of democraticisation and deliberation (*democratic empowerment*). Differentiating between different calls for empowerment on the basis of its underlying political, economical and ethical goals, values, and assumptions helps us to avoid unduly simplistic and binary assessments of whether or not a particular technological practice empowers or disempowers patients. Instead it enables us to carry out a systematic analysis of how technological practices are involved in shifting power between different actors. Applying such an analysis on the field of DTC genetics shows, for example, the great extent to which battles for increasing access to information for patients also enable the types of work that patients are increasingly expected to do in order to make personalised and precision medicine possible.

### EPL1 The evolution of Genetic Counseling

#### EPL1.1

##### Data sharing to support UK clinical genetics and genomics services

**S. Raza<sup>1</sup>, A. Hall<sup>1</sup>, C. Rands<sup>2</sup>, S. Deans<sup>2</sup>, D. McMullan<sup>2</sup>, M. Kroese<sup>1</sup>;**

<sup>1</sup>PHG Foundation, Cambridge, United Kingdom, <sup>2</sup>Association for Clinical Genetic Science, Birmingham, United Kingdom.

Access to high quality data on genomic variants and associated clinical information is fundamental to the delivery of clinical genetics diagnostic services. A complex interplay of practical, technical, legal and regulatory factors are currently impeding the ability of NHS clinical genetics laboratories to share and access the necessary data. Among the most significant challenges are (a) lack of a designated sustainable infrastructure or mechanism for laboratories to share data and (b) inconsistency guidance issued by local NHS Trusts to individual laboratories across the country on what constitutes acceptable practice with regards to the sharing of rare genetic variants which may be considered personally identifiable information. The

PHG Foundation in association with the UK's Association for Clinical Genetic Science, co-hosted a workshop to examine the most pressing challenges around data sharing and to identify priority areas for policy development. Our analysis highlighted that the current arrangements for sharing genomic variants within the NHS is unsatisfactory and inconsistent practices are causing significant difference in patient care and are compromising quality and safety. There is an urgent need for (i) national agreement on the legitimacy of data sharing, (ii) standardised operational processes, including a designated sustainable database or mechanism for sharing, (iii) strong leadership by the multiple relevant health organisations to demonstrate the benefits and risks associated with sharing and not sharing data. In concert these recommendations will go some way towards improving consistency in practice and building trust and confidence amongst patients, the public and healthcare professionals.

Funding from PHGF and ACGS

#### EPL1.2

#### Landscape of genetic tests worldwide: a report from the NIH Genetic Testing Registry (GTR)

A. J. Malheiros, B. L. Kattman, B. Gu, V. Hem, K. S. Katz, M. Ovetsky, R. Villamarín-Salomon, G. Song, C. Wallin, D. R. Maglott, J. M. Lee, **W. S. Rubinstein**; NCBI/NLM/NIH, Bethesda, MD, United States.

The Genetic Testing Registry (GTR; <https://www.ncbi.nlm.nih.gov/gtr/>) at the U.S. National Institutes of Health (NIH), is a freely available resource which enables centralized access to comprehensive information and improve transparency about genetic tests.

Clinical and research genetic test information is voluntarily submitted by providers worldwide. As of February 2016, GTR has 32,903 tests for 5,996 conditions offered by 462 laboratories in 41 countries. The scope includes biochemical, cytogenetic and molecular tests. There are 303 pharmacogenomic tests; 5,568 tests for neoplasms and hereditary cancer syndromes of which 622 tests have somatic targets; and 219 tests for human genome, whole exome and/or mitochondrion.

Of the 8,438 NGS tests, 79% report out only a single gene result. Panels (defined here as having > 4 targets) comprise 16.4% (1,380) of NGS tests. This shows that ordering clinicians can selectively order 1 or more genes performed as part of an NGS assay which helps avoid reporting of markers added to improve sensitivity of the assay but have unclear validity or utility. GTR collects and distributes useful data like laboratory contact information, certifications and staff. Description of clinical tests include conditions, test targets, methods, analytical validity, clinical utility, target population and proficiency testing. Registration of research tests includes study details, methodology and test targets. Submitters must update data annually to ensure that consumers have access to the most up-to-date information. Comprehensive GTR data can be downloaded as XML using FTP or E-Utilities. GTR supplements pages with relevant information including practice guidelines from sources like ACMG and Orphanet.

#### EPL1.3

#### Informing clinical implementation of genomics by "doing" - Practitioner perspectives on integrating genomics in their practice

M. Martyn<sup>1,2</sup>, E. Forbes<sup>1</sup>, A. Kanga-Parabia<sup>1</sup>, I. Macciocca<sup>3</sup>, S. Metcalfe<sup>2,4</sup>, L. Keogh<sup>4</sup>, E. Lynch<sup>1</sup>, The Melbourne Genomics Health Alliance, C. Gaff<sup>1</sup>;

<sup>1</sup>Melbourne Genomics Health Alliance, Parkville, VIC, Australia, <sup>2</sup>Murdoch Childrens Research Institute, Parkville, VIC, Australia, <sup>3</sup>Victorian Clinical Genetics Services, Parkville, VIC, Australia, <sup>4</sup>University of Melbourne, Parkville, VIC, Australia.

**BACKGROUND:** Health professionals' ability to incorporate genomics in practice has been recognised as a barrier to the use of genomics. Building this capability requires more than education alone. As part of a broad clinically-driven program to implement genomics, we aimed to build clinicians' capability by providing them with the opportunity to gain experience of testing patients. Clinicians' views on the impact of their participation and on future service delivery were collected.

**METHODS:** Clinicians offered testing to >300 patients within a demonstration project in Melbourne, Australia. Semi-structured interviews were conducted with 4 genetic counsellors, 14 geneticists and 14 other medical specialists (n = 32) participating in the demonstration project; interviews were analysed using qualitative content analysis.

**RESULTS:** Genetic and other practitioners reported that participating in the demonstration project gave them new insights into how and when to use genomic testing and the changes necessary to support its use in practice. The 'hands-on' learning experience provided by multidisciplinary variant interpretation meetings was particularly valued. Perspectives on future service delivery encompassed processes for patient selection, variant interpretation

and clinic organisation. Results for each will be presented.

**DISCUSSION:** This study confirms that the effective use of genomics in patient care depends in part on a clinical environment which supports this practice. Crucial insights have been gained into aspects of clinical practice which may need to alter to progress the transition to genomic medicine. These results are informing implementation of systems locally that meet the needs of both the workforce and patients.

#### EPL1.4

#### Genetic Counsellor training in the Genomics Era: The development of a new training scheme in England

**M. Bishop<sup>1</sup>, C. Benjamin<sup>2,3</sup>, L. Boyes<sup>4,5</sup>, G. Hall<sup>6</sup>, R. Macleod<sup>6</sup>, M. McAllister<sup>7</sup>, A. Middleton<sup>8</sup>, C. Patch<sup>9</sup>, N. Latham<sup>1</sup>, A. Seller<sup>1,10</sup>, V. Davison<sup>1</sup>, S. Hill<sup>11</sup>,**

<sup>1</sup>Health Education England, Birmingham, United Kingdom, <sup>2</sup>UK and Eire Genetic Counsellor Registration Board Member, Preston, United Kingdom, <sup>3</sup>University of Central Lancashire, Preston, United Kingdom, <sup>4</sup>Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom, <sup>5</sup>Association of Genetic Nurses and Counsellors, Birmingham, United Kingdom, <sup>6</sup>Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, <sup>7</sup>Cardiff University, Cardiff, United Kingdom, <sup>8</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>9</sup>Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>10</sup>Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, <sup>11</sup>Chief Scientific Officer, London, United Kingdom.

A key aim of England's 100,000 Genomes Project is to create a new genomic medicine service for the National Health Service (NHS). Health Education England is responsible for the education and training of NHS staff, and its Genomics Education Programme (GEP) has been tasked to ensure NHS staff are equipped to deliver this world-leading personalised medicine service. One professional group crucial to this delivery is Genetic Counsellors (GCs). Despite the UK having a strong tradition in training GCs, there is no structured NHS training programme or formalised workforce planning. Without sustained training in place, there will be a shortfall of adequately trained GCs to cope with the increasing demand on genomic services. The GEP has worked with leaders in the GC profession to develop a new training programme in Genomic Counselling under the established Modernising Scientific Careers (MSC) framework. MSC is a UK-wide education and training strategy for the healthcare science workforce and includes diverse specialisms such as laboratory clinical scientists and bioinformaticians. This framework seems a natural fit for GCs because of the strong scientific foundation to their clinical practice. This new training programme, due to commence September 2016, comprises a three-year salaried postgraduate-level programme incorporating: an academic MSc in Genomic Counselling with a defined research component; highly structured and nationally specified workplace training emphasising clinical and counselling skills and applied scientific knowledge. By being part of MSC, GC training will be aligned to complementary scientific professions who GCs will work alongside as part of the growing genomic workforce.

#### EPL1.5

#### Ensuring patient centred care in genomics - patients' experiences of the Melbourne Genomic Health Alliance demonstration project

**E. L. Lynch<sup>1</sup>, M. Martyn<sup>1,2</sup>, I. Macciocca<sup>1,2</sup>, S. Metcalfe<sup>2,3,4</sup>, N. Mupfeki<sup>1</sup>, E. Forbes<sup>1</sup>, E. Creed<sup>1,5</sup>, G. Brett<sup>1,6,3</sup>, E. Wilkins<sup>1,5</sup>, D. Bradford<sup>1</sup>, A. Sexton<sup>5</sup>, L. Keogh<sup>1</sup>, L. di Pietro<sup>6</sup>, Melbourne Genomics Community Advisory Group, Melbourne Genomics Health Alliance, C. Gaff<sup>1,4</sup>,**

<sup>1</sup>Melbourne Genomic Health Alliance, Parkville, Melbourne, Australia, <sup>2</sup>Murdoch Childrens Research Institute, Melbourne, Australia, <sup>3</sup>Victorian Clinical Genetics Services, Melbourne, Australia, <sup>4</sup>University of Melbourne, Parkville, Melbourne, Australia,

<sup>5</sup>The Royal Melbourne Hospital, Melbourne, Australia, <sup>6</sup>The Royal Childrens Hospital, Melbourne, Australia, <sup>7</sup>CSIRO, Parkville, Melbourne, Australia, <sup>8</sup>Genetic Support Network Victoria, Parkville, Melbourne, Australia.

**BACKGROUND:** Much research about the concerns and preferences of those having genomic testing is based on the experience of healthy individuals, with little known about patients with genetic conditions. We address this gap by investigating patients receiving genetic counselling and clinical genomic testing for one of five diverse indications.

**METHODS:** At recruitment to the demonstration project, patients elected whether or not to share their genomic data for further research. To explore patient understanding of and concerns about testing, patients were surveyed after a standard length genetic counselling session (<60mins), but before receipt of test results. Descriptive statistics were used to analyse scales and item lists. Content analysis was used for open-ended questions.

**RESULTS:** To date 62% (179/287) have responded. Only 20 (11%) expressed concerns about testing. These primarily related to the familial nature of the result, with few concerns about privacy or security. Most (98%) felt they had received enough information about genomic testing before

making a decision and the majority displayed good knowledge of the test, correctly answering questions about the numbers of genes analysed and expected types of results. Additionally, 93% of patients permitted access to their data for further research unrelated to their condition.

**DISCUSSION:** After genetic counselling, patients concerns about genomic testing are similar those well described for genetic testing. In contrast to healthy research participants undergoing testing, patients with a clinical indication have few concerns about privacy or security prior to receiving results. This is reflected in their decisions to share data broadly for research.

## EPL1.6

### Evolving genetic counselling practice in bicultural New Zealand, a case study of CDH1 testing in a large Māori whānau (family)

**K. K. Gonet;**

Genetic Health Service NZ, Auckland, New Zealand.

Introduction: The current generally accepted model of genetic counselling is based on a person centred approach. Although more emphasis is now placed on cultural competency for genetic counsellors, practice still incorporates a predominantly Western perspective. For this, and a number of historical reasons, there is a lack of confidence and participation in healthcare services by Māori in New Zealand. We worked with one whānau with a CDH1 mutation to address problems with patient engagement and develop a stronger relationship between Māori and the genetics service.

Method: This case study provides the opportunity to explore the evolution of a 'whānau centred' approach to genetic counselling working with Māori in New Zealand. A person centred, western model of genetic counselling includes a focus on individual autonomy and informed consent. In contrast many Māori still hold a collectivist notion of the self, and healthcare decisions are made as a group. By incorporating results of discussions with Māori elders and using the unique Māori health perspectives we developed a mutually agreed upon process of 'Whānau focused' counselling.

Results: By incorporating the Māori health elements of spiritual, psychological, physical and family into genetic counselling a respectful and reciprocal relationship has been established which has resulted in increased uptake of predictive testing. The positive outcomes of this process demonstrate the importance of building strong relationships with different cultural groups. This is a vital aspect of Genetic Counselling and will become increasingly so as genomics leads to more complex information and implications for individuals and whānau.

## EPL2 The Implication for Families of Various Genetic Diseases

### EPL2.1

#### Feedback on professional experiences on the disclosure of genetic information to family members in France

**S. de Montgolfier**<sup>1,2</sup>, E. Rial-Sebag<sup>3</sup>, D. d'Audiffret<sup>4</sup>, C. Farnos<sup>5</sup>, B. Derbez<sup>1</sup>, A. de Pauw<sup>5</sup>, F. Galacteros<sup>6</sup>, D. Stoppa-Lyonnet<sup>7</sup>;

<sup>1</sup>IRIS (Ehess/Inserm), Paris cedex 13, France, <sup>2</sup>Université Paris Est Créteil, Créteil, France,

<sup>3</sup>UMR 1027 Inserm / Université Paul Sabatier, Toulouse, France, <sup>4</sup>Lipha Université Paris-Est Marne-la-Vallée, Marne la Vallée, France, <sup>5</sup>Institut Curie, Paris, France, <sup>6</sup>APHP Hôpital Henri Mondor, Créteil, France, <sup>7</sup>Institut Curie, Inserm U830, Université Sorbonne-Paris-Cité, Paris, France.

The diagnosis of a serious genetic anomaly can be clinically relevant for family members when preventive measures or treatments exist. In France, the legislator introduced in 2011 a specific and original system for disclosing genetic information to family members. Patients have now a legal duty to inform their relatives about genetic risks when it is relevant for their own health. This information can be delivered directly by the patient himself, or indirectly via a procedure involving the prescribing doctor.

We propose to present the results of a survey conducted for three years on the implementation of the French procedure into practices. This was explored across juridical analyses, ethnographical study in genetic services, empirical survey with professionals and patients associations representatives. Firstly, we will present the incidence in terms of responsibility for actors of this new legal procedure; Secondly we will focus on the improvements of practices induced by this legal framework but also on the difficulties that the professionals could meet. These results underline how transmission to family members raises ethical and legal issues, particularly in light of the principles of privacy, confidentiality, autonomy and right to know or not to know in genetic field.

We will end up with some recommendations in the context of information to kin in genetics (role of actors, chronology of events, criteria of genetic

diseases involved, the relevance of information by professionals ...). Research project financed by INCa and the Canceropole Ile-de-France. "Family disclosure in human genetics" (subvention 2013-130)

### EPL2.2

#### Co-designing an intervention to facilitate family communication about inherited genetic conditions (IGC)

**A. Metcalfe**<sup>1</sup>, E. Rowland<sup>1</sup>, I. Eisler<sup>1,2</sup>, M. Ellison<sup>3</sup>, F. Flinter<sup>4</sup>, J. Grey<sup>5</sup>, S. Hutchison<sup>2</sup>, C. Jackson<sup>1</sup>, L. Longworth<sup>6</sup>, R. MacLeod<sup>7</sup>, M. McAllister<sup>8</sup>, T. Murrells<sup>1</sup>, C. Patch<sup>1,4</sup>, G. Robert<sup>1</sup>, F. Ulph<sup>9</sup>;

<sup>1</sup>King's College London, London, United Kingdom, <sup>2</sup>South London and Maudsley NHS Trust, London, United Kingdom, <sup>3</sup>Huntington's Disease Youth Organisation, London, United Kingdom, <sup>4</sup>Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom,

<sup>5</sup>AMEND - Association for Multiple Endocrine Neoplasia Disorders, London, United Kingdom, <sup>6</sup>Brunel University, London, United Kingdom, <sup>7</sup>Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, <sup>8</sup>Cardiff University, Cardiff, United Kingdom, <sup>9</sup>University of Manchester, Manchester, United Kingdom.

Introduction: Parents often want to talk to their children about an Inherited Genetic Condition (IGC) that affects their family. However they are unsure about what to say, when to say it, and are concerned about their child(ren)'s reaction. As a result, children may be given little or no information and they may be afraid of upsetting their parents by asking. The silence that occurs around the IGC can be detrimental to the long-term mental health and well-being of parents and children.

Methods: To facilitate better family communication about the IGC, we co-designed a therapeutic intervention based on Multi-Family Discussion Groups (MFDG) used in systemic family therapy. To inform the intervention's development, a series of focus groups for parents, children, young people and genetic counsellors were held. Genetic counsellors were subsequently trained in the intervention's delivery.

Findings: Training in the MFDG can extend the genetic counsellors role in delivering this intervention. Families and genetic counsellors considered MFDG important for facilitating family communication. Most parents and their children thought families with similar IGC but not necessarily the same should attend the MFDG so that they could relate to and learn from each other's experiences. The sessions should be welcoming environments that simultaneously facilitate involvement in group activities but also provide distraction from the emotionally challenging subject matter. Desired outcomes included: a happier home life, design of a communication tool kit for families' use and the development of informal networks.

Conclusion: The co-designed MFDG's effectiveness will be evaluated in a randomised controlled trial.

### EPL2.3

#### Children's Understanding of Genetics and Inheritance: implications for Genetic Counselling

**M. M. Radu**, R. Moldovan;

Department of Psychology, Babes-Bolyai University, Cluj-Napoca, Romania.

Objectives: Children have naive concepts of heredity primarily based on their daily social interactions. Research exploring children's understanding of heredity and the most adequate approach of genetic mechanisms for them is scarce. The main objective of this research was to explore children's perception of heredity; specifically, we wanted to clarify how children understand heredity and what theories they use in order to understand various genetic mechanisms.

Methods: We collected data using semi-structured interviews with 20 children (11 to 13 years old). The interview was based on 17 questions aimed at exploring their understanding and explanations of heredity. Responses were recorded, transcribed and analyzed using thematic analysis. A 3 year follow up was conducted in order to investigate the dynamic and potential changes in their understanding of heredity and genetic mechanisms.

Results: Following thematic analysis three main themes emerged: (1) inferred mechanisms, (2) inheritance, and (3) misconceptions. Overall, children in this age group have a number of naive theories and mix accurate scientific knowledge with misconceptions, but their general understanding of heredity is rather fair. Sub-themes and specific examples, as well as longitudinal changes in terms of knowledge dynamic are presented.

Discussion: Children have a good understanding of heredity which they integrate in the construct of kinship, though without being able to detail the mechanisms underlying them. The corroboration of our results with the current practice in genetic counselling highlights the implications of this study which is being detailed.

**EPL2.4****Twenty years' experience conducting presymptomatic testing for late-onset neurological diseases: what have we learned?**

M. Panquei<sup>1,2,3</sup>, J. Félix<sup>1,2</sup>, Á. Ménedes<sup>1,2,4</sup>, C. Lemos<sup>1,2,3</sup>, S. Lédo<sup>1,2</sup>, J. Silva<sup>1,2</sup>, J. Sequeiros<sup>1,2,3</sup>,  
<sup>1</sup>IS – Instituto de Investigação e Inovação em Saúde, Univ. Porto, Porto, Portugal,  
<sup>2</sup>UnIGENE and Centre for Predictive and Preventive Genetics (CGPP), IBMC – Institute for Molecular and Cell Biology, Univ. Porto, Porto, Portugal, <sup>3</sup>ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Univ. Porto, Porto, Portugal, <sup>4</sup>Department of Health Sciences, Univ. Aveiro, Aveiro, Portugal.

A national programme of genetic counselling and presymptomatic testing for late-onset neurological diseases was begun, in 1995, at five genetic services in Portugal. It was made accessible to adults at-risk for Machado-Joseph disease, and then extended to other hereditary ataxias, Huntington disease and familial amyloid neuropathy, following a multidisciplinary approach. We aim now at describing the consultands' profile, for a better understanding of the population seeking our services and to reflect on the protocol we have been following for 20 years.

We reviewed 1,330 records of consultands who requested presymptomatic testing at our centre. Their social-demographic profile was similar to those in other international programs. Mean age at the time of testing was 30.5 years; females (55.7%) predominated; 60.5% consultands had no offspring; and 61.4% were non-carriers. Contrarily to other international reports, withdrawals before results disclosure were only about 15%. Among the reasons for uptaking PST, the most common were: relieving uncertainty (41.7%); preparing for the disease-onset (23.2%); family planning (23.20); and informing their offspring (18.0%).

Lessons from our practice along these 20 years point out to the need for harmonization of our registers of counselling sessions, as well as the relevance of a more qualitative approach to document the consultands' experiences. For a better understanding of presymptomatic testing, as we have conducted it until now, we still need tools for quality assessment of counselling practice, as well as the study of at-risk relatives who decide not to be tested and of family-related aspects surrounding uptake of the presymptomatic protocol.

**EPL2.5****Predictive testing for Huntington's disease under the age of 18 years in the UK 1993-2014.**

O. W. J. Quarrell<sup>1</sup>, R. C. Cann<sup>1</sup>, A. Clarke<sup>2</sup>, C. Compton<sup>3</sup>, A. Fryer<sup>4</sup>, S. Jenkins<sup>5</sup>, N. Lahiri<sup>6</sup>, R. MacLeod<sup>7</sup>, Z. Miedzybrodzka<sup>8</sup>, P. J. Morrison<sup>9</sup>, H. Musgrave<sup>10</sup>, M. O'Driscoll<sup>11</sup>,  
<sup>1</sup>Sheffield Children's Hospital, Sheffield, United Kingdom, <sup>2</sup>University Hospital of Wales, Cardiff, United Kingdom, <sup>3</sup>Guy's and St Thomas' NHS Trust, London, United Kingdom, <sup>4</sup>Liverpool Women's Hospital, Liverpool, United Kingdom, <sup>5</sup>University Hospital of Southampton, Southampton, United Kingdom, <sup>6</sup>St George's Hospital, London, United Kingdom, <sup>7</sup>St. Mary's Hospital, Manchester, United Kingdom, <sup>8</sup>University of Aberdeen, Aberdeen, United Kingdom, <sup>9</sup>Belfast City Hospital, Belfast, United Kingdom, <sup>10</sup>Leeds Teaching Hospitals, Leeds, United Kingdom, <sup>11</sup>Birmingham Women's Hospital, Birmingham, United Kingdom.

Background: guidelines for predictive testing for Huntington's disease (HD) were developed in the late 1980's. They became a paradigm for predictive testing in general and have been updated periodically. A consistent feature has been that minors should not be tested, this has always been <18 years in the UK.

Aim: to present information on tests which have been performed on minors in the UK since 1993.

Method: a UK HD Predictive Testing Consortium has collected data annually on the number of HD predictive tests undertaken from 23 genetic centres. The number of cases of predictive testing on minors was identified. Where possible, the circumstances of the test were categorised into broad groups. Results: 9252 tests, with age of testing recorded, were on the database of which 59 (0.64%) were reported on those <18 years. The number of tests at ages: 17, 16, 15, 14, 12, 11 and under 11 years were: 34, 12, 4, 2, 2, 1 and 4 respectively. There were records from 21 out of 23 centres. Details were available for 23 patients. Categories which could be identified included: young person close to age 18 and delay would not add anything; pregnancy in someone <18 years; not testing was judged to cause more harm; young person in a care system and more support would be available <18 years; person never going to be in a position to give informed consent.

Conclusion: we need to understand and monitor the practice of undertaking HD predictive tests on minors.

**EPL2.6****'I've had to fight for everything': a qualitative study exploring the experiences of support of young people with juvenile Huntington's Disease, and their parents, in England**

P. Curtis<sup>1</sup>, O. Quarrell<sup>2</sup>, R. Cann<sup>2</sup>, H. Santini<sup>3</sup>,

<sup>1</sup>University of Sheffield, Sheffield, United Kingdom, <sup>2</sup>Sheffield Children's Hospital NHS Foundation Trust, Sheffield, United Kingdom, <sup>3</sup>Huntington's Disease Association, Liverpool, United Kingdom.

Background: Previous studies examining parents' experiences of caring for a child with juvenile Huntington's disease (JHD) have highlighted their isolation and their need for ongoing support. However, young peoples' own perspectives have not previously been explored and little is known about how support needs might best be met.

Aim: To explore parents' and young people's experiences of support services and their perceptions of how these might be improved.

Methods: The paper presents findings from the initial, qualitative element of a multiphase, mixed-methods study. Multiple strategies (including referrals from clinical genetics centres and from the Huntington Disease Association) were utilised to identify 14 families with a child, aged ≤ 25 years, with JHD. Eight interviews, involving ten parents and young people were undertaken and subjected to Framework analysis.

Findings: Supporting a young person with Huntington's disease requires significant physical, psychological and financial resources. Parents often feel that they have to proactively fight for services and support. Both parents and young people are concerned about professionals' lack of specialist knowledge around juvenile onset Huntington's disease. They are also concerned about the lack of social stimulation and accessible recreational activities for young people.

Conclusion: Parents and young people would benefit from ongoing contact with a knowledgeable key worker to avoid having to repeatedly initiate contact and re-establish relationships with providers as the disease progresses. [Research funded by the NHS National Institute for Health Research. Research for Patient Benefit stream. PB-PG-112-29056]

**EPL3 Incidental Findings and Consent****EPL3.1****Development of a shared clinical exome sequencing consent form across multiple organisations**

I. Macciocca<sup>1,2,3</sup>, Z. Stark<sup>1,3</sup>, D. Bruno<sup>1,3</sup>, J. Taylor<sup>4</sup>, S. M. White<sup>1,3,5</sup>, T. Y. Tan<sup>1,5,3</sup>, G. R. Brett<sup>1,2,3</sup>, E. Creed<sup>1,2</sup>, E. Lynch<sup>2</sup>, C. Community Advisory Group<sup>2</sup>, A. Januszewicz<sup>6</sup>, C. Gaff<sup>2,7,5</sup>, <sup>1</sup>Victorian Clinical Genetics Services, Melbourne, Australia, <sup>2</sup>Melbourne Genomics Health Alliance, Melbourne, Australia, <sup>3</sup>Murdoch Childrens Research Institute, Melbourne, Australia, <sup>4</sup>Royal Melbourne Hospital, Parkville, Melbourne, Australia, <sup>5</sup>University of Melbourne, Melbourne, Australia, <sup>6</sup>Health Legal, Melbourne, Australia, <sup>7</sup>Walter and Eliza Hall Institute, Melbourne, Australia.

Introduction: Informed consent for exome sequencing poses new challenges due to the complexity of the test and the many opportunities for data sharing post-test. As part of the Melbourne Genomics Health Alliance ([www.melbournegenomics.org.au](http://www.melbournegenomics.org.au)), a shared clinical exome sequencing consent form was developed for clinical services and testing laboratories embedded within the ten member organisations of the Alliance.

Method: A working group of representatives from laboratory genetics, medical genetics and genetic counselling from partner organisations was convened to develop the consent form. Consensus was reached on key elements of the form, following review of literature and selected local and international clinical consent forms for exome sequencing. Early consumer input was achieved through the Melbourne Genomics Community Advisory Group. The documents were refined over 10 months and subjected to independent legal review.

Outcomes and conclusion: The outcome was a single page consent form for singleton or trio analysis supported by a series of information sheets describing genomic sequencing and providing an explanation of the points that patients agree to in the consent form.

To date, the consent form has been adopted by two Alliance laboratories and one clinical department and will be used to consent more than 500 patients from five hospitals over the next two years. Up-skilling of health professionals on the use of the consent form is ongoing.

A collaborative approach to the development of an exome sequencing consent form is achievable, enabling a standardised approach to consent across multiple organisations, and facilitating data sharing in an ethically acceptable manner.

**EPL3.2****Genomic investigations and incidental findings: the time for broad consent****G. Crawford<sup>1,2</sup>, A. Fenwick<sup>1</sup>, A. Lucassen<sup>1,2</sup>**<sup>1</sup>University of Southampton, Southampton, United Kingdom, <sup>2</sup>Wessex Clinical Genetics Service, Southampton, United Kingdom.

**Introduction:** Rapidly declining costs and increasing availability of whole-genome analysis mean that broader explorations of the entire genome have replaced targeted approaches to testing. Many debates have been held about adaptations to consent and disclosure practices as these technologies gain widespread use. Possible results include: (a) diagnostic or informative to the clinical reason for testing, (b) diagnostic or informative findings unrelated to the clinical reason for testing (so-called Incidental Findings- IFs) and (c) results of uncertain clinical significance. Here we report the results from an empirical study exploring the challenge these results pose to the consent process.

**Methods:** In-depth interviews were undertaken with 32 health care professionals (HCPs) and 16 patients to explore views about consent and disclosure practices with genomic tests and in particular IFs. These were analysed thematically.

**Results:** Both groups:

1. Agreed that patients should be asked and given choices about the results they wish to receive
2. Realised that discrete choices were not possible since the range of possible findings was too great
3. Wanted clinical judgement to play an important role in disclosure, and were satisfied when this had been superimposed on unclear consent
4. Concluded that broad rather than specific consent was the only type of real consent possible but had concerns about how this could be applied in practice

**Conclusions:** Placing all choices at the consent to testing stage is neither practical nor desirable. Broad consent can provide more valid consent than long and detailed consent.

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**EPL3.3****Outcomes of a Randomized Controlled Trial of Consent Models for Genome Sequencing****B. B. Biesecker<sup>1</sup>, P. Chrysostomou<sup>2</sup>, H. Peay<sup>3</sup>, L. Nelson<sup>2</sup>**<sup>1</sup>NHGRI, NIH, Bethesda, MD, United States, <sup>2</sup>NICHD, NIH, Bethesda, MD, United States,<sup>3</sup>RTI, Chapel Hill, NC, United States.

Consent to undergo genome sequencing is more complex than consenting participants to a single gene test. The depth and scope of the results and dimensions of uncertainty exceed any prior testing. No standard for consent to participate in genome sequencing studies exists. This randomized controlled trial (RCT) compares an evidence-based streamlined consent (4 pages) to a "standard" NIH consent (6 pages). Outcomes were knowledge, informed choice and decisional conflict. Randomization to one of the models was followed by a consent discussion with a genetic counselor. Participants completed surveys at baseline, directly following the consent intervention and six weeks later. Two hundred twelve women affected with primary ovarian insufficiency (POI) and eligible for an exome study participated in the RCT; 192 completed all three surveys. The women were on average 39.2 years old ( $\pm 6.8$  years), white (78.8%) and held a college (34.8%) or post-graduate (51.1%) degree. There were no statistically significant differences in knowledge between the consent models ( $F=0.93$ ,  $p=0.336$ ). Most participants had a greater understanding of the benefits and limitations of genome sequencing but less understanding of hereditary concepts. Patients with a family history of POI (30) had a significantly better understanding of sequencing ( $r=0.16$ ,  $p=0.031$ ) than those with no family history (139). If informed choice and decisional conflict complement our knowledge results, our findings suggest a streamlined consent may be as effective as a standard NIH consent process.

**EPL3.4****The UK 100,000 genomes project: views, expectations, and experiences of the first patients recruited****S. Dheensa, A. Lucassen, A. Fenwick, G. Crawford**

University of Southampton, Southampton, United Kingdom.

**Introduction:** The 100,000 genomes project is unprecedented and introduces several practices that are novel for the health service. As well as seeking a genetic diagnosis for the presenting rare disease, patients and families can have their genomes interrogated for additional risks amenable to intervention, such as inherited cancers and cardiac conditions. Notably, testing for autosomal-recessive carrier risk is offered solely to couples planning to

have more children and reported only if both parties are carriers. We are exploring views, expectations, and experiences of this new venture, and in particular, consent and communication practices for additional and carrier findings.

**Methods:** Our research design is mixed-methods, involving questionnaires and longitudinal interviews with participating patients and families; focus groups with healthcare professionals; and observations of consent and feedback consultations. At abstract submission, we had analysed 65 questionnaires and 15 interviews.

**Results:** Patients/families have realistic expectations about receiving a primary diagnosis. However, they also have limited understanding of the information materials provided. They are unsure what genetic and genomic tests involve; are anxious about when, how, and by whom results will be delivered; are apprehensive about the uncertain implications of primary and additional findings; and although feel privileged to have additional testing, would prefer to receive individual carrier results.

**Conclusions:** Patients/families speak positively of whole-genome sequencing. Nevertheless, we are identifying areas of the consent process that warrant improvement. To this end, we will design digital tools based on our ongoing research to help families understand their results and, where relevant, communicate them to relatives.

**EPL3.5****Diagnostic whole exome sequencing in pediatrics: Comparing parents' pre- and post-disclosure attitudes toward return of results****C. Cornelis<sup>1,2</sup>, A. Tibben<sup>3</sup>, W. Dondorp<sup>4</sup>, M. van Haelst<sup>1</sup>, A. Bredenoord<sup>5</sup>, N. Knoers<sup>1</sup>, M. Duijwell<sup>2</sup>, I. Bolt<sup>2</sup>, M. van Summenen<sup>6</sup>**<sup>1</sup>Department of Genetics, University Medical Center Utrecht, Utrecht, Netherlands,<sup>2</sup>Ethics Institute, Utrecht University, Utrecht, Netherlands, <sup>3</sup>Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, <sup>4</sup>Department of Health, Ethics & Society, Maastricht University, Maastricht, Netherlands, <sup>5</sup>Julius Center, Department of Medical Humanities, University Medical Center Utrecht, Utrecht, Netherlands, <sup>6</sup>Department of General Pediatrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, Netherlands.

**Introduction:** Parents' experiences, attitudes, and preferences regarding return of results from diagnostic whole exome sequencing (WES) for their child remain largely unexplored. Yet these insights are needed for morally responsible policy-development.

**Method:** Semi-structured interviews were conducted with parents of 16 children at two moments: parents were first interviewed after consenting to WES (trio-analyses), but prior to feedback of results; parents were then interviewed again after receiving the results.

**Results:** Some parents reported changes in attitudes toward disclosure of certain findings while waiting for results, but none expressed a desire to modify choices for unsolicited findings (UFs) or to revoke their decision for WES. Parents received different types of results or combinations thereof: primary results and/or UFs - some results were of unclear significance. Experiences with disclosure of results varied and depended largely on what parents' reasons were for consenting to WES and/or receiving UFs as well as on the expectations and hopes on which their reasoning was based. Sometimes results were in line with parent's expectations or positively exceeded them; sometimes expectations were only partially fulfilled, for example, this included situations in which an important reason for consenting to WES was to envision the child's future (cognitive) development, but where results only offered a general indication of what level of development the child might be able to attain.

**Conclusion:** These insights inform ethical theorizing on policy-development for WES and UFs in child cases.

This project is funded by ZonMw (grant 70-73000-98-047) - The Netherlands Organization for Health Research and Development.

**EPL3.6****Who is my family's keeper? Professional and family ethics in the era of unsolicited findings****R. H. P. Wouters<sup>1</sup>, E. E. Voest<sup>2</sup>, R. M. Bijlsma<sup>1</sup>, M. G. E. M. Ausems<sup>1</sup>, J. J. M. van Delden<sup>1, A. L. Bredenoord<sup>1</sup></sup>**<sup>1</sup>University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>The Netherlands Cancer Institute, Amsterdam, Netherlands.

**Introduction:** as next-generation sequencing finds its way into clinical practice, ethical issues regarding disclosure of genetic information towards family members become more urgent than ever before. Who is responsible for conveying genetic risk information to family members? Unsolicited findings put geneticists and counsellors in a dilemma between (amongst others) respecting their patients' privacy and providing potentially life-saving information to probands' relatives. In this debate the focus has particularly been on professional moral duties, and patients' and family members' own

responsibilities are easily being overlooked.

Materials and Methods: this presentation provides an ethical analysis of professionals' and patients' responsibilities towards family members at-risk for curable or preventable genetic diseases. Arguments from different ethical perspectives will be identified and critically evaluated.

Results: the duty to warn is equally demanding to genetic professionals and patients. Yet professionals have conflicting duties such as patient confidentiality. Responsibilities that arise within a family do not play an important role in contemporary medical practice, but the current debate on unsolicited findings draws the attention towards the patient as moral agent co-responsible for his children's and siblings' health.

Conclusion: there is a moral basis for the claim that both patients and professionals have a duty to warn relatives that are at risk for hereditary diseases. New strategies for genetic disclosure as a shared responsibility are needed. In order to achieve this, IT applications may provide a valuable contribution. This research is funded by the Dutch Cancer Society.

## EPL4 Reporting the Results: Clinical and Ethical Considerations

### EPL4.1

#### When children become adults: should biobanks re-contact?

N. A. A. Giesbertz<sup>1,2</sup>, A. L. Bredenoord<sup>2</sup>, J. J. M. van Delden<sup>2</sup>;

<sup>1</sup>Department of Genetics, UMC Utrecht, Utrecht, Netherlands, <sup>2</sup>Department of Medical Humanities, Julius Center, UMC Utrecht, Utrecht, Netherlands.

Pediatric biobanks facilitate research, which is considered important for improving (pediatric) health care by generating biomedical knowledge. However, pediatric biobank research gives rise to specific ethical issues. At the time of inclusion, many children cannot, or are legally not allowed to, consent for themselves, and typically parental permission is required. Samples may still be stored and used by biobanks when children become autonomous adults. The question arises whether children should be re-contacted to obtain their own consent, or give the opportunity to withdraw their samples, when they reach adulthood. Major guidelines do not provide sufficient guidance on re-contact and consent, and there is only very limited literature that analyses the issue in depth. Given the fact that biobanks already include pediatric samples, and in light of the rapid developments in biobank research, it is important to address the issue of re-contact and consent now. We discuss arguments in favor and against re-contacting participants at maturity and examine different re-contact policies that can be considered, ranging from a thin opt-out policy (participants can withdraw their samples, but the biobank does not re-contact the participant) to a strict opt-in (samples will be destroyed when participants do not give their consent). We suggest that biobanks adopt a thick opt-out as the default re-contact policy, which means that biobanks re-contact children at maturity and give them the opportunity to withdraw their samples.

### EPL4.2

#### Re-contact in clinical practice: investigating the perspectives of healthcare professionals in the United Kingdom

D. Carrieri<sup>1</sup>, S. Dheensa<sup>2</sup>, S. Doheny<sup>3</sup>, P. D. Turnpenny<sup>4</sup>, A. J. Clarke<sup>3</sup>, A. M. Lucassen<sup>2</sup>, S. E. Kelly<sup>1</sup>;

<sup>1</sup>Egenis, University of Exeter, Exeter, United Kingdom, <sup>2</sup>Faculty of Medicine, University of Southampton, Southampton, United Kingdom, <sup>3</sup>School of Medicine, Cardiff University, Cardiff, United Kingdom, <sup>4</sup>Royal, Devon & Exeter Hospital, Exeter, United Kingdom.

The rapid introduction of genetic technologies in medicine is creating new information that can have implications for patients' and families' health, reproductive decisions, lifestyle choices, employment, and psychosocial wellbeing. Do healthcare professionals, such as clinical genetics specialists, have a responsibility or duty to re-contact former patients to communicate this new information? There is no professional consensus about whether and how re-contacting should happen. There is also limited empirical evidence concerning the perspectives of healthcare professionals and patients. We conducted interviews with over 30 healthcare professionals from clinical genetics, and other specialties potentially involved in re-contacting. Re-contact does take place, but generally in an *ad hoc* fashion, e.g. when clinicians happen to review a file for other reasons. Decisions about whether and how to re-contact were based on the specific patient and/or family, and the perceived utility of the result. Whilst there was uncertainty about whether more systematic re-contacting processes should be implemented, the need for a professional debate to agree in what situations re-contacting should be considered good standard of practice was highlighted. Some re-

spondents argued that the creation of re-contact guidelines may help clinical services to be allocated more resources. Others argued that this might create a legal obligation to re-contact thereby using up scarce resources. To determine whether guidelines would be useful, more empirical evidence is needed. To this aim, we will also explore the experiences and expectations of patients and other stakeholders, such as support groups.

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### EPL4.3

#### Incidental findings derived from Next-Generation sequencing: what does actionable in childhood really mean?

A. Laberge<sup>1</sup>, J. Richer<sup>2</sup>;

<sup>1</sup>Medical Genetics, Department of Pediatrics, Ste-Justine, Montreal, QC, Canada, <sup>2</sup>Children Hospital of Eastern Ontario, Ottawa, ON, Canada.

Genome-wide sequencing has been performed on a research basis for several years and now clinical diagnostic services based on these technologies are increasingly requested. Incidental findings are identified in 1-2% of tested individuals undergoing whole exome sequencing. The question of which incidental findings to return to patients has generated much discussion including formal position statements in Canada, the USA and Europe. While most agree that actionable findings should be offered to all competent adults, the discussion becomes more complex for paediatric patients with a number of organizations recommending reporting back incidental findings actionable in paediatric. In order to better understand which conditions may be actionable in childhood, we approached the question from a public health perspective, applying the WHO screening criteria to determine if a condition was a good target for opportunistic screening in the context of genome sequencing. We applied this framework to the list of 56 genes conditions considered actionable and therefore reportable by the ACMG in the context of genomic sequencing. We concluded that assessment of actionability during childhood should strongly consider issues of:

- o penetrance of treatable manifestations (not simply of any manifestation of the condition) at 18 years,
- o likelihood and severity of adverse outcome with this condition,
- o whether a clear risk-reduction intervention with proven (not simply anecdotal) benefits is available.

For incompletely penetrant conditions, the likelihood to have a deleterious impact on the patient if we wait for them to display clinical symptoms to establish a diagnosis should also be considered.

### EPL4.4

#### Autonomy in the genomics era

A. J. Newson;

University of Sydney, Sydney, Australia.

Introduction: Genomic testing is placing unprecedented pressure on the concept of autonomy. Traditionally a paramount principle in genetic testing, autonomy is now at risk of being trumped by other considerations, such as what may be in an individual's or population's 'best interests'; or the ongoing normalisation and integration of genomic information in health care.

Materials and Methods: This paper will use methods of theoretical bioethics (applied ethics). That is, the paper will proceed by way of analysing key concepts and case studies to build a normative ethical position on the role that considerations of autonomy should play in genomics.

Results: The paper will commence with an analysis of autonomy and how it has traditionally been employed in clinical genetics and associated disciplines. Then, using case studies, an apparent move away from presumptions of autonomy will be described. This emergent status will then be critiqued, to build an argument that autonomy should remain at the heart of genomic decision-making. However, it will also be recognised that the concept of autonomy may need refinement; in particular to account for the potential ubiquity and ongoing shared nature of genomic information. The goal is to resolve tensions between individual and collectivist approaches to information provision and decision-making. Conclusions: The paper will conclude by claiming that autonomy is not an obsolete concept and that it has significant relevance for genomic medicine. This will mean that emerging practices, such as an inability to opt out of receiving genomic information, may not always be ethically defensible.

## EPL4.5

### An exploration of reporting practices for next generation sequencing technologies with laboratory personnel

D. F. Years<sup>1</sup>, K. Sénécal<sup>2</sup>, P. Borry<sup>1</sup>;

<sup>1</sup>Centre for Biomedical Ethics and Law, KU Leuven, Leuven, Belgium, <sup>2</sup>Centre of Genomics and Policy, McGill University, Montreal, QC, Canada.

Next-generation sequencing (NGS) technologies are identifying causative mutations in individuals who would have previously remained undiagnosed, influencing their treatment and providing important information to their families. However, they also have the potential to identify additional genomic information that is unrelated to their illness, such as variants in genes with unknown function (VUS) and mutations in genes causing phenotypes extraneous to the clinical question (incidental findings, or IFs). Recommendations by professional bodies outlining which findings should be reported by laboratories to the clinician requesting the test are inconsistent. Little is known about which variants laboratories are reporting and how they are using guidelines to make decisions about this.

To address these questions, in-depth interviews were conducted with laboratory staff in Europe, Canada and Australia to explore reporting practices for diagnostic NGS. Reporting of variants of uncertain significance (VUS) differed between laboratories depending on the perception of its relevance to the clinical question. There was considerable variation in the filtering strategies that participants described. While some laboratories use quite stringent filtering in order to limit the number of IFs that are identified, others are less strict, allowing identification of these variants. Whether these IFs are then reported to the clinician often involves detailed discussions, involvement of multidisciplinary committees, and consideration of factors such as actionability.

Our study highlights that laboratories are still grappling with decisions about which variants to report from NGS. These findings will assist laboratories to learn from each other's experiences and refine reporting guidelines.

## EPL4.6

### Informing preparation for personal genomic screening

J. Fleming<sup>1</sup>, B. Terrill<sup>2</sup>, M. Dziadek<sup>2</sup>, E. Kirk<sup>2</sup>, A. Roscioli<sup>2,3</sup>, K. Barlow-Stewart<sup>4</sup>;

<sup>1</sup>University of Sydney Medical School - Northern, Sydney, Australia, <sup>2</sup>Garvan Institute of Medical Research, Sydney, Australia, <sup>3</sup>Sydney Children's Hospital, Randwick, Sydney, Australia.

Exploring the experience of individuals undertaking personal genome screening (PGS), offered in Australia since 2014, provides a unique opportunity to inform preparation for testing. To date 15 individuals have been interviewed: nine with genetics expertise, six without. PGS results included an autosomal dominant condition neurofibromatosis type 1 (NF1) not previously clinically identified; carrier status for recessive condition(s); a number of variants identified as likely pathogenic but many of uncertain significance; and pharmacogenetically relevant mutations.

Analysis has identified common themes between the groups: The importance of the pre-testing counselling session with positive experiences with clinical geneticists/genetic counsellors. Barriers to uptake: including skepticism of family members, medical practitioners, genetics colleagues, and privacy concerns. Genetics professionals cited rationale for testing as professional interest and/or curiosity, without anticipating personal or family impact; non-genetics professionals reported personal interest, curiosity, and interest in being an early adopter. On reflection, despite this initial objective motivation, several found the impact of the test results had unanticipated personal impact and changed over time and later recognized their relevance, as health problems developed or family history was interrogated more closely. Disclosure of results has been limited. Participants felt that expectations; residual risk; changes in interpretation with developing phenotypes; and personal and family impact and communication needed greater emphasis at the pre-test session. Some non-genetics professionals felt information provided in the report was too complex.

As saturation of data from genetic professionals has been achieved, interviewing of non-genetics professionals is continuing to further explore similarities and differences between the two groups.

## EPL5 From Public Understanding to Educating Professionals

### EPL5.1

#### Socialising the Genome

A. Middleton<sup>1</sup>, J. Borrà<sup>2</sup>, K. Nevin-Ridley<sup>2</sup>, V. Parry<sup>2</sup>, A. Sanders<sup>4</sup>, J. Rayner<sup>5</sup>;

<sup>1</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>2</sup>Thin Air Factory, London, United Kingdom, <sup>3</sup>Genomics England, London, United Kingdom, <sup>4</sup>Wellcome Trust, London, United Kingdom, <sup>5</sup>Wellcome Genome Campus, Cambridge, United Kingdom.

How to start a conversation about genomics with people who may know nothing about it? This is particularly relevant for patients involved in the 100,000 Genomes Project, a landmark new UK sequencing project taking place within the National Health Service. We aimed to 'socialise' genomics for participants and their families using an innovative method that combines evidence from social science with creative story telling as used by the advertising industry. We have done this through the use of animations designed to get patients talking with their families or health professionals. Through insights gained from five focus groups with membership drawn from the British public, we were able to develop core themes on which to base our animations. Using the creative skills of a senior advertising director from an established advertising company the themes were turned into six narratives. These delivered information about genomics using metaphors and cultural context, as opposed to scientific text or drawings. Feedback on each of the animations will be presented from a representative British public group of 500 people as well as from 1,000 participants and publics. The six novel, evidence-based animations will be used as part of a public engagement campaign for Genomics England. We will show our open access animations in our presentation. They are freely available for anyone to use, irrespective of where they live in the world.

### EPL5.2

#### General public's attitudes towards genetics and genetic testing

D. Chokoshvili<sup>1</sup>, C. Belmans<sup>1</sup>, R. Poncelet<sup>1</sup>, S. Sanders<sup>1</sup>, D. Vaes<sup>1</sup>, I. Huys<sup>1</sup>, P. Borry<sup>1</sup>, University of Leuven, Leuven, Belgium.

As genetic testing becomes more readily available, it is important to study attitudes of the general public towards human genetics and genetic testing. To address these questions, we performed structured interviews with visitors to the annual cartoon festival in Knokke-Heist, Belgium, during the summer of 2014. The main theme of the festival was challenges and progress in human genetics and more than 100 000 visitors attended the event. The survey was completed by 1182 respondents, resulting in a demographically diverse sample with a mean age of 48.5 years (range 16-87). The majority of respondents (64.6%) were curious about their genetic predisposition to diseases, while 49.5% were interested in testing solely for treatable and/or preventable disorders. Among the respondents aged 41 or younger (n=399), 54.6% were willing to undergo carrier screening for recessive disorders before pregnancy and 54.1% would consider testing their unborn children for all serious genetic diseases during pregnancy. Almost two-thirds (64.7%) of all participants anticipated that in the near future, genetic tests will be routinely required by insurance companies for determining the insurance premium, while 23.9% indicated they were worried that genetic tests results would fall in the wrong hands. The concern over potential misuse of genetic test results was significantly greater among those who identified as religious (p<0.01). Studying the opinions and concerns of the general public is essential to ensure socially responsible adoption of new genomic technologies and identify the areas where educating the public could offer the greatest benefits.

### EPL5.3

#### Exploring Australian public knowledge and understanding of genetic concepts and terminology in the era of personal genomics

S. A. Metcalfe<sup>1</sup>, B. Terrill<sup>2</sup>, C. Hickerton<sup>1</sup>, J. Savard<sup>3</sup>, E. Turbill<sup>1</sup>, A. Newson<sup>3</sup>, C. Gaff<sup>4</sup>, K. Gray<sup>5</sup>, A. Middleton<sup>6</sup>, B. Wilson<sup>7</sup>;

<sup>1</sup>Murdoch Childrens Research Institute and Dept Paediatrics, University of Melbourne, Parkville, Vic, Australia, <sup>2</sup>Garvan Institute of Medical Research, Sydney, NSW, Australia,

<sup>3</sup>The University of Sydney, Sydney, NSW, Australia, <sup>4</sup>Walter and Eliza Hall Institute of Medical Research and Dept Paediatrics, University of Melbourne, Parkville, Vic, Australia, <sup>5</sup>Health and Biomedical Informatics Centre, University of Melbourne, Parkville, Vic, Australia, <sup>6</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>7</sup>School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa, Ottawa, ON, Canada.

Personal genomic testing provides healthy individuals with access to information about their genetic makeup for purposes that include ancestry, paternity, sporting ability and health. While much of this testing has occurred within the USA, it is likely that such testing will become routine and acces-

sible to everyone.

Focus groups were conducted within a multi-disciplinary project to explore Australians' awareness of personal genomic testing. In mid-2015, 56 members of the public participated in 7 focus groups, allocated into 3 age groups: 18-25, 26-49 and ≥50 years. Three researchers coded transcripts independently and themes were generated. Here we present themes focusing on awareness of personal genomic testing and genetic literacy.

No-one had heard of the term 'direct-to-consumer' testing; most were not familiar with 'personal genomic testing' as such, but could deduce what 'personal genomics' might entail. Participants' descriptions of DNA, genetics and genomics varied according to prior experience and education. They were familiar with ideas of heredity and acknowledged genetic influences on physical characteristics. There were diverse perceptions of the relative influence of genetics and environment on health, mental health, behaviour, talent or personality. While many participants felt their own understanding of genetics was limited and did not mention specific terms, such as epigenetics, they were able to contribute to discussions using descriptive language. The focus groups highlighted challenges of using specific, jargon-laden genetic terms in public discussions. These findings can inform development of more engaging services and educational resources for the public to understand and make decisions around these marketed tests.

#### EPL5.4

#### Development of Test Ordering Recommendations for Clinicians with Minimal Genetics Background from the ClinGen Consortium Consent and Disclosure Recommendations (CADRe) Committee

K. E. Ormond<sup>1</sup>, M. Hallquist<sup>2</sup>, A. Buchanan<sup>2</sup>, M. Cho<sup>1</sup>, K. Brothers<sup>3</sup>, C. R. Coughlin II<sup>4</sup>, L. Hercher<sup>5</sup>, L. Hudgins<sup>4</sup>, S. Jamal<sup>6</sup>, H. Levy<sup>7</sup>, H. Peay<sup>8</sup>, M. Roche<sup>9</sup>, M. Stosic<sup>10</sup>, M. Smith<sup>11</sup>, W. Uhlmann<sup>12</sup>, K. Wain<sup>13</sup>, W. A. Fauci<sup>2</sup>;

<sup>1</sup>Stanford University School of Medicine, Stanford, CA, United States, <sup>2</sup>Geisinger Health System, Danville, PA, United States, <sup>3</sup>University of Louisville, Louisville, KY, United States,

<sup>4</sup>University of Colorado, Aurora, CO, United States, <sup>5</sup>Sarah Lawrence College, Bronxville, NY, United States, <sup>6</sup>Sick Kids Hospital, Toronto, ON, Canada, <sup>7</sup>Johns Hopkins University, Baltimore, MD, United States, <sup>8</sup>Parent Project Muscular Dystrophy and DuchenneConnect Registry, Research Triangle, NC, United States, <sup>9</sup>University of North Carolina, Chapel Hill, Chapel Hill, NC, United States, <sup>10</sup>Natera, San Carlos, CA, United States, <sup>11</sup>Northwestern University, Chicago, IL, United States, <sup>12</sup>University of Michigan, Ann Arbor, MI, United States, <sup>13</sup>Geisinger Medical Center, Danville, PA, United States.

Molecular genetic testing is increasingly ordered by clinicians in specialties beyond clinical genetics. Germline genetic testing can involve complex ethical, legal, social, medical and logistical (ELSIplus) issues; non-genetics clinicians have limited training in adjusting communication plans for patients based on these issues. Requiring all patients undergoing genetic testing to be referred for traditional genetic counseling is not scalable. Therefore, this project developed rubrics to guide non-genetics clinicians in identifying potential consent and disclosure ELSIplus issues that may inform the consent/disclosure approach and degree to which genetics clinicians may be involved for a given gene. We recruited stakeholders representing viewpoints of genetic counselors, MD geneticists, ethicists, pediatricians, policy experts, and patient advocates to join CADRe and convened multiple conference calls and an in-person meeting. We then used snowball sampling methodology to survey clinicians to assess whether the rubric was effective. We will present the rubric development and revision process, during which 14 participants enumerated many topics to consider, including: test and disease characteristics, provider/patient misinterpretation, growing cultural awareness of genetic testing in the general public, patient preferences, varying quality and availability of educational materials, necessity for a follow-up plan, cascade testing, testing context, emotional burden, and lab/clinician reporting methods. Survey results will demonstrate the rubric use for genes including HTT, DMD, OTC, GJB2, MLH1, CDH1, TP53, CYP2C9 and VKORC1. The CADRe committee expects to further revise the rubrics after focus groups are conducted in summer 2016, and plans to provide review of actionable genes using the CADRe rubric during 2017.

#### EPL5.5

#### E-learning to improve communication about cancer family history and knowledge on hereditary colorectal cancer by non-genetic health professionals

K. F. L. Douma, E. M. A. Smets, E. Dekker, P. J. Tanis, C. M. Aalfs;  
Academic Medical Center, Amsterdam, Netherlands.

Introduction: A recent study showed that hereditary colorectal cancer often goes unrecognized, because of inadequate discussion of cancer family history. This leads to suboptimal referral for genetic counselling and can have negative health consequences for both patients and family members. To improve knowledge and discussion of cancer genetic topics we aim to develop

an e-learning for gastroenterologists (GEs) and surgeons. Materials and methods: Through an online focus group we investigated the attitudes of 3 GEs and 5 surgeons in training towards cancer family history collecting, discussing genetic testing and their needs regarding an e-learning intervention. Subsequently, the results were discussed with a GE, surgeon, clinical geneticist, medical psychologist, an educational and an information technology expert to develop a framework. Results: GEs and surgeons in training are positive towards collecting a family history. However, they lack experience and oncogenetic knowledge and perceive this as more important than lack of communication skills. Furthermore, they perceive time pressure as well as difficulties in managing patient's reactions. Also, they lack knowledge on useful information sources (e.g. guidelines). These clinicians appreciate an e-learning, but want it to be short and case-based. Conclusions: The focus group has shown that the e-learning should be short, case-based and focus on knowledge instead of communication skills. Currently, the e-learning is tested among a group of surgeons and GEs (n=50). The e-learning and its evaluation will be presented during the conference.

This study is financially supported with a Dutch Cancer Society fellowship grant (UVA 2011-4918)

#### EPL5.6

#### Onco-equip: Preparing healthcare professionals in cancer care for routine genetic testing

L. M. Jackson, H. Skilton;  
Plymouth University, Plymouth, United Kingdom.

Genetic testing can now inform management options for individuals affected with breast or ovarian cancer and will therefore be increasingly used in cancer care settings. The aim of this project was to prepare medical and nursing staff who are involved in offering and performing genetic testing prior to making cancer management decisions. The key objective was to ensure that testing is offered safely and appropriately to patients in Europe. We used an expert advisory group consisting of clinicians, scientists and patient representatives to define the required content of an educational programme and developed a series of four online interactive modules. These are: how genes influence breast and ovarian cancer, genetic testing in patients with cancer, consent for genetic tests and explaining the result. Each module is based on a case scenario and learners must interact with the material to progress through the module. This educational programme can be undertaken free of charge and has been widely advertised using websites, Twitter, Facebook and at relevant professional conferences. A pre and post module test is part of the package, and professionals who wish to can obtain a certificate of completion after achieving a post-module score of at least 80%. Initial pre-module scores indicate that many health professionals working in these settings have poor understanding of the topic and that there are significant differences in knowledge as a result of the programme. Here we report further on the development of the educational program and the initial educational outcomes.

#### EPL6 Helicopter View On Cancer Genetics

##### EPL6.1

#### Genetic Counselling Preferences and Psychological Impact of the Analysis by Next-Generation-Sequencing in Clinical Oncology (PIANO study)

I. Esteban<sup>1</sup>, F. Balaguer<sup>2</sup>, E. Adrover<sup>3</sup>, E. Carrasco<sup>1</sup>, N. Gadea<sup>1</sup>, M. Vilaró<sup>1</sup>, G. Llort<sup>4</sup>, R. Jovert<sup>5</sup>, M. Herráiz<sup>6</sup>, S. Kohrrami<sup>7</sup>, A. Herreros de Tejada<sup>8</sup>, R. Morales<sup>9</sup>, J. Cano<sup>10</sup>, R. Serrano<sup>11</sup>, B. Graña<sup>12</sup>, C. Guillén<sup>13</sup>, J. Alés<sup>14</sup>, J. Brunet<sup>15</sup>, J. Balmáñia<sup>16</sup>;

<sup>1</sup>Vall d'Hebron Hospital, Barcelona, Spain, <sup>2</sup>Hospital Clínic Barcelona, Barcelona, Spain, <sup>3</sup>Hospital Universitario General de Albacete, Albacete, Spain, <sup>4</sup>Hospital Sabadell-Parc Taulí, Barcelona, Spain, <sup>5</sup>Hospital General de Alicante, Alicante, Spain, <sup>6</sup>Hospital Clínico Universidad de Navarra, Pamplona, Spain, <sup>7</sup>Hospital Son Espases, Palma de Mallorca, Spain, <sup>8</sup>Hospital Universitario Puerta de Hierro, Madrid, Spain, <sup>9</sup>Hospital La Mancha Centro, Alcázar de San Juan, Spain, <sup>10</sup>Hospital General de Ciudad Real, Ciudad Real, Spain, <sup>11</sup>Hospital Reina Sofía, Córdoba, Spain, <sup>12</sup>Hospital Abente y Lago, La Coruña, Spain, <sup>13</sup>Hospital Ramón y Cajal, Madrid, Spain, <sup>14</sup>Hospital de Nuestra Señora de Sonsoles, Ávila, Spain, <sup>15</sup>Heredity Cancer Program, Catalan Institute of Oncology, Girona, Spain.

Introduction: Multiplex cancer testing allows for parallel sequencing simultaneously, but little is known about the psychological impact of these tests. Our aim was to analyze the patient's preferences regarding genetic counseling and the psychological impact of a multiplex cancer panel.

Materials and Methods: 210 patients who underwent panel testing (MyRisk 25-gene panel, FAMOSA study) were included (76% women, 44% with prior negative genetic result). Participants completed self-questionnaires regarding genetic counseling preferences and three psychological scales (MICRA, CWS, R-IES) at baseline, one week and three months after results disclosure.

**Results:** Most participants would like to be disclosed variants of unknown significance (VUS) and deleterious variants in moderate penetrance genes (72% and 78%, respectively).

At one week, mutation carriers showed higher distress compared to non-carriers and VUS carriers ( $p<0.01$ ). Adverse reactions were higher in those with positive results (7.56) than those with VUS (4.86) and those with negative results (3.44) ( $p<0.001$ ), and this difference was maintained after three months ( $p<0.01$ ). Participants who carried a mutation in a moderate penetrance gene showed higher cancer worry than high-penetrant mutation carriers ( $p<0.05$ ). No differences in uncertainty were observed, regardless of the genetic test result.

Overall, the psychological impact (IES score) decreased significantly after 3 months ( $p<0.001$ ).

**Conclusions:** Patients are willing to be disclosed all available information from panel testing. Differences in terms of family support and communication were observed among type of results at short and mid-term. Cancer worry was higher in moderate-penetrance carriers than high-penetrance carriers. Longer follow up is undergoing.

#### EPL6.2

##### The impact of predictive genetic testing for cancer on young adults

*L. Godino<sup>1,2</sup>, D. Turchetti<sup>2</sup>, L. Jackson<sup>1</sup>, C. Hennessy<sup>1</sup>, H. Skirtton<sup>1</sup>,*

<sup>1</sup>School of Nursing and Midwifery, Faculty of Health and Human Sciences, Plymouth University, Plymouth, United Kingdom, <sup>2</sup>Unit of Medical Genetics, Department of Medical and Surgical Sciences, St. Orsola-Malpighi Polyclinic, University of Bologna, Bologna, Italy.

Predictive genetic testing should involve a considered choice, which is particularly true when testing is undertaken in early adulthood. We carried out a systematic review that showed that many young adults grew up with little or no information concerning their genetic risk and that parents had exerted pressure during the testing decision-making process. However, none of the studies retrieved were conducted in Italy or other South-European countries. To address this gap, we undertook a qualitative study based on grounded theory to explore the psychosocial implications of predictive testing for hereditary cancer in young Italian adults aged 18-30 years. Interviews were conducted on three occasions: one month before genetic counselling, two weeks after counselling and six months later. Interviews were transcribed and analysed using grounded theory. To date, a total of 37 interviews with 15 participants have been conducted. Findings are reported under four themes: knowledge, genetic counselling process, decision making and dealing with test results. Although participants grew-up with little or no information about their genetic risk, none expressed regret at having the test at a young age. Pre-test counselling was appreciated as a source of information, rather than a support in decision-making. Decisions were often autonomous and sometimes conflicted with parents' wishes. Participants who have completed all three interviews to date reported changing their health behaviours after learning their genetic test result. Further analysis are required to determine how young adults conceptualise and utilise genetic risk in their daily life to inform genetic counselling practice in this client group.

#### EPL6.3

##### How to approach all high risk members in known Lynch Syndrome families? Experiences of different contact methods and perceived challenges in passing the information to family members in Finland

*K. I. Aktan-Collan<sup>1</sup>, A. Haukkala<sup>1</sup>, E. Koltola<sup>1</sup>, J. Mecklin<sup>2</sup>, K. Pylyvanainen<sup>2</sup>, H. Kääriäinen<sup>3</sup>,*

<sup>1</sup>University of Helsinki, Helsinki, Finland, <sup>2</sup>Jyväskylä Central Hospital, Jyväskylä, Finland, <sup>3</sup>National Institute of Health and Welfare, Helsinki, Finland.

**Introduction:** Identification of hereditary predisposition to cancer has limited significance if not followed by efficient cancer prevention. Of all known 3000 Finnish LS family members tested, 1500 are mutation carriers with 2110 offspring. Of these, 973 have had genetic testing while 1137 not. Most subjects have been contacted using family mediated approach (FMA) via index-patient but we have also contacted them directly (DCA) in research setting. Despite the efforts, 528 known high-risk adults have not been approached, and may be developing a fatal disease. Here, we investigated the challenges involved, and will discuss options for the future.

**Methods:** In DCA study we approached 286 adults with a 50% carrier risk in 102 LS families by direct letter and compared the response with 446 corresponding subjects using FMA. Furthermore, we investigated by questionnaire among carrier parents over age 40 ( $n = 248$ ) needs and challenges in communication to offspring. Qualitative interviews were done among 10 carriers.

**Results:** The test uptake was 75% in FMA and 39% in DCA. One reason for

DCA higher failing may be the contact letter, being too considerate and thus vague. In family communication, informing the children about their risk was experienced most difficult, and 30% of parents reported a wish for professional help. The qualitative interviews indicated that communication did occur but had several complexities.

**Conclusions:** The challenge is to improve the contact and communication processes, so that all family members would get the information important for their healthcare. Possibilities of additional strategies will be discussed.

#### EPL6.4

##### Increasing awareness of lifestyle recommendations for cancer prevention among Lynch syndrome mutation carriers: results of a randomized controlled trial

*A. Visser<sup>1</sup>, A. Vrielink<sup>1</sup>, M. Hoedjes<sup>2</sup>, E. Kampman<sup>3</sup>, N. Hoogerbrugge<sup>1</sup>,*

<sup>1</sup>Radboud university medical center, Nijmegen, Netherlands, <sup>2</sup>Department of Health Sciences, VU Amsterdam, Amsterdam, Netherlands, <sup>3</sup>Radboud university medical center and Wageningen University, Nijmegen and Wageningen, Netherlands.

**Introduction:** Lynch Syndrome (LS) carriers may potentially reduce their cancer risk by adhering to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) lifestyle recommendations for cancer prevention. This study tested the effect of providing LS carriers with WCRF-NL materials on awareness of and adherence to these recommendations.

**Materials and Methods:** A randomized controlled trial in 218 LS carriers was conducted. Two weeks after randomization, the intervention group ( $n=113$ ) received WCRF-NL health promotion materials. All participants were asked to fill out questionnaires at three time points: at baseline (T0), at 4 weeks (T1) and 6 months (T2) after randomization. Per-protocol analyses ( $n=198$ ), using repeated measures ANOVAs, were performed to test differences over time between both groups on the primary outcomes: awareness of (scale 0-7) and adherence to the WCRF/AICR recommendations. Secondary outcomes were knowledge (scale 0-7), cancer risk perception, psychological distress and cancer worry.

**Results:** At T1, the intervention group was significantly more aware ( $M_{intervention} = 5.8 \pm 1.6$ ;  $M_{control} = 4.2 \pm 2.3$ ,  $p<.001$ ) and showed a significantly improved knowledge of the recommendations ( $M_{intervention} = 4.6 \pm 1.7$ ;  $M_{control} = 2.5 \pm 1.6$ ,  $p<.001$ ) compared to the control group. No effect was found on cancer risk perception, psychological distress, or cancer worry. Results on adherence and follow-up results will be presented.

**Conclusions:** The WCRF-NL materials are an easy-to-implement tool to increase awareness of and knowledge about the lifestyle recommendations for cancer prevention among LS carriers, without causing additional distress or cancer worry. Creating awareness is an important first step in increasing adherence to these recommendations.

Grant: WCRF

#### EPL6.5

##### Group-based patient education (GPE) courses for hereditary breast and ovarian cancer

*W. Listøl<sup>1,2</sup>, H. Høberg-Vetti<sup>1,2</sup>, G. Eide<sup>3,4</sup>, C. Bjørvatn<sup>1,5,6</sup>,*

<sup>1</sup>Western Norway Familial Cancer Center, Haukeland University Hospital, Bergen, Norway, <sup>2</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, <sup>3</sup>Centre of Clinical Research, Haukeland University Hospital, Bergen, Norway, <sup>4</sup>Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway, <sup>5</sup>Learning and Mastery Center in Bergen, Bergen, Norway, <sup>6</sup>Department of Clinical Science, University of Bergen, Bergen, Norway.

**Introduction:** Women carrying *BRCA*-mutations are facing significant challenges, including decision making regarding risk-reduction. They often report that they are left alone with these important decisions. In order to enhance the genetic counselling session, we organized a GPE-course for women with *BRCA*-mutations. In the present study we investigated i.a. the characteristic of those with increased levels of anxiety and depression.

**Materials and Methods:** A prospective study was conducted. Two weeks before (T1) and two weeks after (T2) attending the GPE-course the participants received questionnaires by mail. We collected information on demographic- and medical variables, anxiety and depression, self-efficacy and coping style. Five courses were arranged. A total of  $N = 100$  (77 %) women answered the questionnaires at baseline, and 75 (58%) completed both questionnaires.

**Results:** The mean level of anxiety was quite high among the participants, but was significantly reduced during the follow up period. One of the main findings was that lower levels of anxiety is associated with the time since disclosure of gene test result, higher levels of self-efficacy, and the loss of a close relative due to breast- or ovarian cancer. While lower levels of de-

pression is associated with higher levels of education, and the loss of a close relative due to breast- or ovarian cancer.

**Conclusion:** Overall the women in this study benefited from the course. The most vulnerable women were those newly diagnosed with a *BRCA* mutation, lower levels of self-efficacy and lower levels of education. These women need special attention.

## EPL7 Breaking News

### EPL7.1

#### Development of new resources to improve communication in genetic counselling practice

*M. Alvarez Estapé<sup>1,2</sup>, I. Cuscó<sup>2,3,4</sup>, L. Pérez-Jurado<sup>2,3,4</sup>, C. Serra-Juhé<sup>2,3,4</sup>,*

<sup>1</sup>*Biosciences Faculty, Universitat Autònoma de Barcelona, Bellaterra, Spain, <sup>2</sup>Genetics Unit, Universitat Pompeu Fabra, Barcelona, Spain, <sup>3</sup>Hospital del Mar Research Institute (IMIM), Barcelona, Spain, <sup>4</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Barcelona, Spain.*

**Introduction:** Genetic counselling aims to help patients and families to decide the course of action which seems more appropriate to them and make the best possible adjustment in relation to the genetic disease affecting them. This objective requires, among others, that the counsellor succeeds in communicating in a clear and appropriate manner to the patient and family the information related to the disorder. Many factors interfere in how much information do the counselees absorb and retain and being in an emotionally stressful situation might be one of them.

**Methods:** We identified some common weak points in counselling sessions and some concepts which genetic counsellors at two different hospitals in Barcelona consider complex and important for the families to understand their specific disease.

**Results:** We aimed to develop didactic materials to help counsellors improve the counselees understanding of key concepts in a genetic counselling setting. This material distinguishes the concept of what a chromosome, a gene and the DNA are in clear slides that allow counselees to easily understand these terms. We designed drawings to use in pedigrees hence the patient or family can follow the explanation without getting lost with squares and circles and an explanation of the different types of mutations has also been developed.

**Conclusions:** These support slides and animations offered in Catalan, Spanish and English provide a modern way of communicating genetics by using parallelisms, examples and simplifications with nice and attractive drawings and diagrams which in consequence may help improve patients and families comprehension.

### EPL7.2

#### What determines decision making in preconception carrier screening and can it be influenced with message framing and narrative information?

*J. S. Voorwinden<sup>1</sup>, A. H. Buitenhuis<sup>1</sup>, E. Birnie<sup>1</sup>, A. M. Lucassen<sup>2</sup>, M. A. Verkerk<sup>1</sup>, I. M. van Langen<sup>1</sup>, M. Plantinga<sup>1</sup>, A. V. Ranckor<sup>1</sup>,*

<sup>1</sup>*UMCG, Groningen, Netherlands, <sup>2</sup>University of Southampton, Southampton, United Kingdom.*

**Introduction:** Next-generation sequencing made it possible to create a population based preconception carrier screening test that covers 50 severe autosomal recessive diseases simultaneously. Before this test can be offered, it is important to understand what factors are related to decision making and how the general public can be informed but not influenced in their intention to participate. This will be studied by measuring to what extent message framing and adding narrative information can influence people's intention. **Materials and Methods:** Data was collected by means of online questionnaires among 504 potential users. Factors were based on the Theory of Planned Behaviour and previous research about decision making. Message framing was manipulated by explaining the risk of carriership in different ways, while for narrative information half of the participants received additional narrative information next to factual information.

**Results:** Factors related to more intention are: more perceived benefits about testing, experience the choice to participate as easy, non-religious, more perceived susceptibility of carriership, more knowledge about preconception screening and being female. Message framing and narrative information have no significant effect on intention to participate, perceived susceptibility and perceived severity.

**Conclusions:** This research clarifies which factors are most related to decision making. Also that message framing and the addition of narrative information can inform the general public about preconception carrier screening

but will not influence their participation. As perceived benefits of testing are most related to participation, future research should try to understand how people could be made more aware of these benefits.

### EPL7.3

#### Transparency in the marketing of direct-to-consumer genetic tests

*J. A. Hall<sup>1</sup>, J. E. Amato<sup>2</sup>, C. Pagliari<sup>3</sup>,*

<sup>1</sup>*Vivactiv Ltd, Bellingdon, United Kingdom, <sup>2</sup>Post-operative Intensive Care Unit Staff, Unimed-Rio Hospital, 22775-003, Rio de Janeiro, Brazil, <sup>3</sup>University of Edinburgh Medical School, Edinburgh, United Kingdom.*

**Introduction:** Direct-To-Consumer Genetic Testing (DTCGT) is increasingly marketed to UK consumers, but the transparency of vendors' sales messages or terms and conditions (T&C) is unclear. We analysed information on vendors' websites to assess compliance with Human Genetics Commission recommendations (2010), and sentiments evoked amongst potential consumers.

**Methods:** Companies advertising in the UK were identified through web searches. Accessible T&C and privacy policies, including data re-use, were assessed against HGC benchmarks. Sentiments evoked by the marketing messages were examined through social media consultation.

**Results:** After excluding genealogy and paternity services, 14 companies remained, of which 10 were registered in the UK and subject to UK Data Protection laws. These tested nutri-genetics/lifestyle traits (13/14), pre-symptomatic disease (6/14), drug responsiveness (4/14) and carrier status (2/14). The scientific rationale for gene selection was absent in 9 and only 4 clearly stated all genes to be analysed.

Four companies shared both aggregated and individual-level genetic data for unspecified research. Only 2 offered a separate opt-in for research using individual-level data.

Two companies included pre-test counselling and two post-test counselling (as an upgrade). In the T&C, all companies stated information was non-medical and did not guarantee quality.

Marketing sentiments emphasised performance optimisation, self-improvement, knowledge enhancement, scientific altruism, and personalised medicine.

**Conclusions:** Most DTCGT companies marketing to UK customers are UK registered. Most have lengthy, legalistic T&Cs that consumers are likely to skip. Few HGC recommendations have been adopted. We provide suggestions for increasing transparency, and mechanisms to support oversight of marketing and T&Cs.

### EPL7.4

#### Genomic Newborn Screening: Public Health Policy Considerations and Recommendations

*M. C. Cornel<sup>1</sup>, J. M. Friedman<sup>2</sup>, A. J. Goldenberg<sup>3</sup>, K. J. Lister<sup>4</sup>, K. Sénéchal<sup>5</sup>, D. F. Vears<sup>6</sup>, the Global Alliance for Genomics and Health Regulatory and Ethics Working Group Paediatric Task Team;*

<sup>1</sup>*Section Clinical Genetics, Department of Clinical Genetics and EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands,*

<sup>2</sup>*Department of Medical Genetics, University of British Columbia and Child & Family Research Institute, Vancouver, BC, Canada, <sup>3</sup>The Center for Genetic Research Ethics and Law, Department of Bioethics, Case Western Reserve University, Cleveland, OH, United States, <sup>4</sup>Office of Population Health Genomics, Public Health Division, Department of Health, Government of Western Australia, Perth, Australia, <sup>5</sup>Centre of Genomics and Policy, Department of Human Genetics, McGill University, Montreal, QC, Canada, <sup>6</sup>Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium.*

Genomic technologies such as genome-wide sequencing, can identify genetic causes of rare paediatric diseases much more effectively than conventional clinical and laboratory methods. However, the nature of this technology and its potential to identify additional information about the child being tested raises a range of concerns, particularly when these technologies are applied in a public health setting, such as newborn screening (NBS).

The Global Alliance for Genomics and Health is an international collaboration of more than 370 healthcare, research, disease advocacy, life science, and information technology institutions formed to promote human health through sharing of genomic and clinical data [<http://genomicsandhealth.org/>]. Within this remit, the Paediatric Task Team of the Global Alliance's Regulatory and Ethics Working Group was established to address issues of particular relevance to child health.

This Paediatric Task Team has developed the following recommendations for clinicians, clinical laboratory scientists, and policy makers regarding the use of genomic technologies for population-based newborn screening: NBS by genomic methods should only be considered as an add-on to current screening programs, which should not be replaced unless equal or better

sensitivity and specificity is shown. Equal availability and accessibility to every infant born in the jurisdiction should be guaranteed. Data sharing is needed for interpretation of variants. Publicly-funded universal newborn screening by genomic methods should be limited to diseases that can be effectively treated or prevented early in life. A program should guarantee treatment and follow up. We conclude we are not yet ready to implement sequencing large multigene panels in NBS.

## EPL7.5

### Cancer genetic counselling based on electronic mega-pedigrees incorporating Cancer Registry information

V. Stefansdottir<sup>1,2</sup>, O. T. Johannsson<sup>3</sup>, H. Skirtton<sup>4</sup>, L. Tryggvadottir<sup>5,6</sup>, J. J. Jonsson<sup>1,7</sup>;

<sup>1</sup>Dept. of Genetics and Molecular Medicine, Landspitali - University Hospital, Reykjavik, Iceland, <sup>2</sup>Dept. of Biochemistry and Molecular Biology, Univ. of Iceland, Reykjavik, Iceland, <sup>3</sup>Dept. of Medical Oncology, Landspitali - University Hospital, Reykjavik, Iceland, <sup>4</sup>Plymouth University, Plymouth, United Kingdom, <sup>5</sup>Icelandic Cancer Registry, Reykjavik, Iceland, <sup>6</sup>Faculty of Medicine, University of Iceland, Reykjavik, Iceland, <sup>7</sup>Dept. of Biochemistry and Molecular Biology, Univ. of Iceland, Reykjavik, Iceland, Reykjavik, Iceland.

Cancer risk assessment is initially based on pedigree information. In Iceland, cancer genetic counselling is undertaken by constructing mega-pedigrees using information from a large genealogy database and the population-based Icelandic Cancer Registry. This enables rapid identification of counselees that belong to families with a known BRCA variation. Since January 2007, over 1900 individuals with concerns about familial breast and ovarian cancer have been seen.

Until recently, only two BRCA founder variations were known in the Icelandic population i.e. the *BRCA2:c.771\_775del5* with carrier frequency 0.6-0.8% and the *BRCA1:5193G>A*, with unknown but low frequency. Through the cancer genetic service, three other *BRCA1* and one *BRCA2* pathogenic variations have been found in six families.

Up to eight pedigrees are made for counselees: those of the father and mother of each grandparent and their descendants. This method enables accurate tracing of any mutation. A mega-family is defined as a pedigree not sharing carrier individuals with another family. Pedigree size (mega-family) can vary from 50-4400 individuals (average 379). Up to the end of December 2015, 455 families have been traced. Sixty-two families with *BRCA2 c.771\_775del5* variation and four with *BRCA1:5193G>A* variation.

In all, 1400 counselees have been tested for an alteration in a BRCA gene. Of 755 individuals tested for *BRCA2:c.771\_775del5*, 339 had a positive result, while of 58 tested for *BRCA1:5193G>A*, 27 were positive. Families have a positive attitude about the use of mega-pedigrees. This approach may be useful in other countries where there is access to genealogical databases.

## EPL7.6

### Genetic testing for osteogenesis imperfecta on children suspected of abuse: does testing put parents at greater risk?

E. Youngblom, D. J. Bowen, P. H. Byers, P. Pecora, L. Kelly;  
University of Washington, Seattle, WA, United States.

Introduction: Non-accidental injury (NAI, or abuse) is a worldwide pediatric concern. The prevalence of NAI with fractures is 2.4/1,000 in the 0-3 year rage. Osteogenesis imperfecta (OI), which is characterized by fragile bones, is the most common genetic condition confused with NAI (prevalence 0.1/1000). Genetic testing can be used to help distinguish OI from NAI.

Materials and Methods: To understand how genetic test results for OI are used by professionals involved in child protection, one-on-one semi-structured telephone interviews were conducted with 5 child welfare workers and 5 child abuse lawyers (dependency side) that were known to have some experience or involvement with this issue. Interviews were recorded, transcribed, and coded by two separate coders.

Results: Interview results revealed that these professionals are not typically trained about causes of skeletal injury besides NAI, and that testing might be limited by financial, policy, or guidelines limitations. When genetic testing is done, results are sometimes misunderstood and thus misrepresented in court in the hands of professionals untrained in genetic analysis.

Discussion: While genetic testing can identify children with OI among those investigated for NAI, testing could also come at some risk. Misunderstanding of genetic test results in the hands of a non-geneticist professional could result in serious ramifications for family members, and improved guidance and training may be necessary to avoid unintended adverse consequences. Further exploration of these issues will be conducted by surveying a larger population of these professionals later this year.

## ESY1 Symposium Diversity

### ESY1.1

#### Improving access to genomic medicine projects by underserved populations: exemplars from USA, Australia and UK

N. Qureshi<sup>1</sup>, S. Metcalfe<sup>2</sup>, B. Julian<sup>3</sup>, M. Parker<sup>4</sup>, V. Bonham<sup>5</sup>, E. Lynch<sup>6</sup>, N. Khan<sup>7</sup>;

<sup>1</sup>University of Nottingham, Nottingham, United Kingdom, <sup>2</sup>University of Melbourne, Melbourne, Australia, <sup>3</sup>University of Leicester, Leicester, United Kingdom, <sup>4</sup>University of Oxford, Oxford, United Kingdom, <sup>5</sup>National Human Genome Research Institute, Bethesda, MD, United States, <sup>6</sup>Melbourne Genomics Health Alliance, Melbourne, Australia, <sup>7</sup>Manchester Centre for Genomic Medicine, Manchester, United Kingdom.

Internationally, population-based genomic studies are being rolled out, for example, the Genome England 100,000 project. Previous studies have failed to engage underserved population, such as, minority communities and those from lower socioeconomic backgrounds.

Presenters will introduce their respective organisation's strategies to improving recruitment and retention of participants from underserved populations. Broadly, considering equality of access, reducing stigmatisation and appropriate patient information on recruitment and during the study.

Considering international experience:

USA:Community input is critical to the successful implementation of National Human Genome Research Institute's genomics research programs in clinical medicine. Through consortiums such as Implementing Genomics in Practice, Clinical Sequencing Exploratory Research and Population Architecture using Genomics and Epidemiology, NHGRI supports funding of research that includes the objective of enhancing the participation of diverse populations. These initiatives will be presented.

ENGLAND: The risk of recessive disorder is increased in consanguineous families. In these families there is little awareness of inherited disorders and research has identified that lack of knowledge, poor communication, language barriers and stigma are all factors inhibiting access. In Manchester a multidisciplinary strategy integrating clinical, educational and community engagement strands have been implemented to enhance access to genetic services. Similarly, East of England Genomic Medicine Centre have developed innovative approaches to tackling inequality of access to genomic medicine in particular access to familial cancer services.

AUSTRALIA: Melbourne Genomics Health Alliance (MGHA), comprising 10 organisations (hospitals, research institutes and a university), has a vision of integrating genomics in everyday healthcare by establishing a single shared approach to genomic information across these multiple organisations, for the benefit of patients, clinicians and researchers. English as second language (ESL) was not a barrier for recruitment to the project, with 9% of patients having ESL and at least 18 languages spoken. Genetic counsellors used either formally trained hospital interpreters or family members for the consent process. Genetic counsellors noted various issues with use of interpreters, including concerns about privacy, logistical issues, inaccurate interpretation and cultural issues.

### ESY1.2

#### Engaging diverse populations in genomics research and reducing inequalities in the implementation of genomic medicine

V. L. Bonham;

National Human Genome Research Institute, Bethesda, MD, United States.

As we advance our understanding of the interaction between gene expression and disease, genomic medicine has the potential to dramatically improve health outcomes. However, as genomic medicine becomes the standard of care and is integrated into personalized and precision treatments, the current lack of ancestrally diverse participants in genomics research creates a risk of exacerbating existing health disparities in underrepresented populations.

We will highlight three exemplary models of engagement of underserved and ancestrally diverse populations in genomics research in the United States. We will also report on the United States National Institutes of Health, National Human Genome Research Institute (NHGRI) roundtable convened in 2015 to discuss the opportunities and challenges associated with the inclusion and engagement of underrepresented populations in genomics research.

## ESY1.3

### Experience from Melbourne Genomics Health Alliance to improve access for underserved population

*E. L. Lynch<sup>1</sup>, S. Metcalfe<sup>2,3</sup>, E. Kowal<sup>4</sup>, M. Martyn<sup>1,2</sup>, E. Forbes<sup>1</sup>, Melbourne Genomics Health Alliance, C. Gaff<sup>1,3</sup>;*

<sup>1</sup>*Melbourne Genomic Health Alliance, Melbourne, Australia, <sup>2</sup>Murdoch Children's Research Institute, Melbourne, Australia, <sup>3</sup>University of Melbourne, Melbourne, Australia, <sup>4</sup>National Centre for Indigenous Genomics, Canberra, Australia.*

(\*Joint First Authors) A major aim of the Melbourne Genomics Health Alliance demonstration project was to understand patients' experiences of having genomic sequencing and their preferences for testing in the future. Melbourne's population is amongst the fastest growing and most culturally diverse in Australia. Overall, English was a second language (ESL) for 9% of patients enrolled in the project, speaking 18 different languages. We identified some problems accessing experiences and preferences of ESL patients regarding genomic testing, which have informed strategies to engage better with ESL patients in Phase 2 of Melbourne Genomics. Another project in Australia is the National Centre for Indigenous Genomics (NCIG) which aims to establish a national resource, under Indigenous governance, for appropriate and respectful genomic research that will benefit Indigenous Australians. NCIG has developed policies and procedures to provide a model for genomic research with Indigenous people globally and has also commissioned a short animation for Indigenous Australians explaining scientific concepts underpinning DNA collection.

Medical records were also reviewed to triangulate the data collected across three different media to add rigour.

Results: Participants reported having little or no experience with genetic services. Many themes identified were unexpected, such as: (i) family history was unknown, (ii) family communication was hindered by a lack of information and knowledge about the condition and (iii) genetics support was desired. The key results were the lack of awareness about: the condition, its implications for the family and how information and guidance can be accessed from the genetics department.

Conclusion: This study reflects the findings from previous studies regarding the minimal genetic service usage, misconception of genetic information and the complexities surrounding family communication. Additionally, this study has obtained new information about the type of information this population is seeking, barriers to accessing genetic services and desired support.

## EMP1.03

### The role of palliative healthcare professionals in providing access to clinical genetics services: barriers and suggestions for the post-Jolie era

*A. Dearing<sup>1,2</sup>, N. Taverner<sup>2</sup>;*

<sup>1</sup>*St George's Hospital, London, United Kingdom, <sup>2</sup>Cardiff University, Cardiff, United Kingdom.*

Introduction: Palliative healthcare professionals (PHCPs) frequently do not refer their eligible patients to the All Wales Medical Genetics Service. After the death of the affected individual, clinically relevant information for family members is lost. In previous research, PHCPs stated that the end-of-life setting is not appropriate to discuss genetic issues. It is unclear if this has changed due to increasing awareness of genetics in the media and efforts to mainstream genetics.

Materials and Methods: Semi-structured interviews of PHCPs were analysed by thematic analysis.

Results: Seven PHCPs (four nurses, two consultants, and one clinical psychologist) were interviewed. Participants reported feeling unfamiliar with the role of clinical genetics services, and did not feel confident in addressing genetic issues with their patients. A lack of scientific knowledge and unawareness of existing infrastructure to support their patients were cited. Many stated that palliative patients are interested in exploring a potential hereditary component to their disease, and acknowledged the potential for psychological benefit for their patients and their families. Most stated that addressing genetics fits within their skill set, but expressed concern about issues of consent, logistical difficulties, and ethical dilemmas.

Discussion: These perceptions differ considerably from those reported in existing literature. Importantly, each participant stated that the potential benefits of addressing genetic issues outweighed the potential for harm in most cases. These results suggest a need for clinical genetics staff to develop closer links with their local PHCPs and to provide education. Clinical psychologists may also be a helpful resource to address PHCPs' concerns.

## EMP1.04

### Need for multidisciplinary approach in Ehlers-Danlos syndrome: the University Hospital Ghent experience

*V. Szymczak, F. Malfait;*

*Center Medical Genetics, Ghent, Belgium.*

Introduction: Ehlers-Danlos syndrome (EDS) is a group of disorders which affect the connective tissue that support the skin, bones, blood vessels, and many other organs and tissues. The latest classification recognizes six subtypes, including the hypermobility type, which is an autosomal dominantly transmitted disorder with variable expressivity and reduced penetrance. Lifelong follow-up in a multidisciplinary team is needed since the underlying genetic defect has thus far not been identified and hence laboratory diagnosis is currently not available.

Material and Methods: The hypermobility type of EDS may affect as many as 1 in 10.000 people. In 2014, the Ghent University Hospital has launched its multidisciplinary EDS clinic. The team consists of a geneticist, a genetic counsellor, physiotherapists, an occupational therapist, podiatrists, a psychologist and a social worker. After having been diagnosed by the geneticist, patients receive appointments with members of the team, according to their needs. An individual yearly follow-up scheme is offered to each patient.

Results: Since the start of the clinic approximately 300 patients were recorded. Each patient has an individual file where the overall health status is maintained and that is evaluated in a monthly multidisciplinary meeting.

Conclusion: The EDS hypermobility type comprises a clinically heterogeneous group of connective tissue diseases. Since there is no cure, a multidisciplinary approach is required to provide a preventive and symptomatic care. Organizing an EDS clinic where patients are seen on a regularly individual base, at one moment by several experienced specialists, is an added value in optimizing the care.

## EMPAG POSTERS

### EMP1 EMPAG Posters

#### EMP1.01

##### An exploration of circumstances surrounding the continuation of tested pregnancies found to be at high risk of Huntington's disease - implications for clinical practice

*F. Wadrup<sup>1</sup>, S. Holden<sup>1</sup>, A. Clarke<sup>2</sup>;*

<sup>1</sup>*Clinical Genetics, Addenbrooke's Hospital, Cambridge, United Kingdom,*

<sup>2</sup>*Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom.*

In its first 20 years the UK Huntington's Disease Prediction Consortium identified 22 pregnancies at high-risk of Huntington's Disease that were continued following prenatal testing. Huntington's Disease is a severe, inherited neurodegenerative condition. Prenatal testing is available through direct or exclusion methods, but is not indicated for couples committed to completing the pregnancy if the fetus is found to be at a high-risk. This ethically complex scenario ultimately removes the child's future right to make an autonomous decision regarding predictive testing. To explore the circumstances surrounding these 22 cases the genetic centres involved were asked to review the case notes and complete a questionnaire. 14 were returned. Thematic analysis identified time pressure and external influence as two major factors contributing to the couples' decision-making. The inability to predict which couples this will affect was highlighted, as were the barriers to maintaining contact and follow-up. Most commonly the couples involved were described as long-term and committed, and their decision-making confused and emotional. This study suggests genetic practitioners must fully prepare couples for the prenatal test and possible termination of pregnancy experience, including the chance of delays and of new emotions surfacing at each stage. Effective liaison with other health professionals is also essential to ensure awareness of the complexity and implications of the situation.

#### EMP1.02

##### Diagnosed with autosomal dominant polycystic kidney disease: unmet needs and suggested support

*S. T. Carter<sup>1</sup>, M. R. Davies<sup>2</sup>, A. Clarke<sup>1</sup>;*

<sup>1</sup>*Institute of Medical Genetics, Cardiff University, Cardiff, United Kingdom,*

<sup>2</sup>*University Hospital of Wales, Cardiff, United Kingdom.*

Introduction: A review of the literature suggests a lack of research exploring autosomal dominant polycystic kidney disease in the field of genetic counselling. Previous studies focused on the treatment, management, pathophysiology or molecular genetics. This study focused on the affected individuals' experiences with access to genetics services, genetic risk discussions within the family and desired genetic information support.

Methods: A written questionnaire containing both open and closed questions was completed (n = 16). A subset of participants (n=9) completed a semi-structured interview using a pre-determined topic guide. The interview transcripts, and parts of the questionnaire were analysed thematically. Themes were identified if they were expressed by at least half the participants.

**EMP1.05****Exploring the aspects of genetic counseling from non-medical healthcare providers in 8 Genetic Centers in Belgium***S. De Nobele<sup>1</sup>, D. F. Vearns<sup>2</sup>;**<sup>1</sup>Center For Medical Genetics, University Hospital, Ghent, Belgium, <sup>2</sup>Center for Biomedical Ethics and Law. Department of Public Health and Primary Care. KU, Leuven, Belgium.*

**Background.** With the advent of new genetic technologies and the subsequent advances in genetic medicine, non-medical healthcare providers are being enlisted to assist in clinical genetics services. In Belgium, although the professional title of 'genetic counselor' was recently accepted, no formal genetic counseling courses exist. A working group of 'genetic counselors' was established in 2015 to provide support for this growing profession in Belgium. However, the scope of this new role remains unexplored in the Belgian context.

**Objectives.** This study aims to systematically identify the scope of non-medical healthcare providers practices in Belgium.

**Methods.** A survey will be administered to all non-medical healthcare providers working within the eight genetic centers in Belgium in March 2016. Questions will be based on those by Skirton et al. 2013, exploring the aspects of genetic counseling that are relevant to their current practice. We anticipate responses from all 15 non-medical healthcare providers currently working in Belgium.

**Results.** The analysis will focus on 1) participants' knowledge, skills and attitudes regarding psychosocial aspects, and medical and human genetics, 2) the ethical, legal and social issues they face, and 3) professional practice, education and research. Results will be compared to the European situation. **Conclusions.** By understanding the scope of the genetic counseling work performed by non-medical healthcare providers in Belgium, the working group can better assist these individuals in their practice, assess the need for additional training/education programs and provide recommendations for support within the healthcare system.

**EMP1.07****Family history and perceived risk of diabetes, cardiovascular disease, cancer and depression***M. Vornanen<sup>1</sup>, H. Konttinen<sup>1</sup>, H. Kääriäinen<sup>2</sup>, S. Männistö<sup>2</sup>, V. Salomaa<sup>2</sup>, M. Perola<sup>2</sup>, A. Haukkala<sup>1</sup>;**<sup>1</sup>University of Helsinki, Helsinki, Finland, <sup>2</sup>National Institute for Health and Welfare, Helsinki, Finland.*

**Introduction:** Family history is an inexpensive tool to assess risks of multifactorial diseases. However, better knowledge is needed on how family history is related to perceived risks of such diseases. We examined how family history relates to perceived risk of diabetes mellitus, cardiovascular disease (CVD), cancer, and depression, and whether these associations are independent of or moderated by sociodemographics, health behavior/weight status (smoking, alcohol consumption, physical activity, BMI [kg/m<sup>2</sup>]), or depressive symptoms.

**Methods:** Participants were Finnish 25–74-year-olds (N=6258) from a population-based FINRISK 2007 study. Perceived absolute lifetime risks (1–5) and first-degree family history of CVD, diabetes, cancer and depression, and health behaviors were self-reported. Weight and height were measured in a health examination.

**Results:** Family history was most prevalent for cancer (36.7%), least for depression (19.6%). Perceived risk mean was highest for CVD, lowest for depression. Association between family history and perceived risk was strongest for diabetes ( $\beta=0.34$ ,  $P<0.001$ ), weakest for depression ( $\beta=0.19$ ,  $P<0.001$ ). Adjusting for sociodemographics, health behavior, and depressive symptoms did not change these associations. The association between family history and perceived risk tended to be stronger among younger than among older adults, but similar regardless of health behaviors or depressive symptoms.

**Conclusions:** Association between family history and perceived risk varies across diseases. Among those with healthy and unhealthy behavior, family history contributes equally to perceived risk. Future research should seek to identify the most effective strategies to combine familial and genetic risk communication in disease prevention.

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**EMP1.08****What do women want? Asking healthy Italian women about BRCA mutations and ovarian cancer risk management options***M. Franiuk<sup>1</sup>, T. Gavaruzzi<sup>2</sup>, A. Tasso<sup>3</sup>, L. Battistuzzi<sup>4</sup>, L. Varesco<sup>1</sup>, L. Lotto<sup>2,5</sup>;**<sup>1</sup>Unit of Hereditary Cancer, IRCCS San Martino-IST Istituto Nazionale Ricerca sul Cancro, Genoa, Italy, <sup>2</sup>Department of Developmental Psychology and Socialization, University of Padova, Padova, Italy, <sup>3</sup>Department of Human Sciences, University of Ferrara, Ferrara, Italy, <sup>4</sup>Department of Internal*

*Medicine and Medical Specialties, University of Genova, Genoa, Italy, <sup>5</sup>Center for Cognitive Neuroscience, University of Padova, Padova, Italy.*

**Introduction:** Prophylactic oophorectomy in women with BRCA mutations significantly reduces their risk of developing ovarian cancer. While in countries such as the US as many as 70% of BRCA-positive women choose to have this type of preventive surgery, anecdotal evidence suggests that frequencies in Italy are much lower. Our aim was to investigate healthy Italian women's attitudes toward ovarian cancer risk management options, and to identify predictors of the preference for surgery over intensified surveillance.

**Methods:** Healthy women, aged 30 to 45, were asked to a) imagine they had a family history of breast and ovarian cancer and a BRCA mutation they could inherit; b) read informational material on predictive BRCA testing; c) fill out a questionnaire assessing knowledge, risk perception, preferences for cancer risk management options, and socio-demographic variables. Binary logistic regression models were used to identify predictors of the preference for surgery over intensified surveillance.

**Results:** Surveillance was viewed very positively and was the preferred option, whereas surgery was chosen by 24% of the 181 participants. Predictors of choice were associated with: knowledge (knowing that life expectancy is longer with surgery compared to surveillance, perceived comprehension of the consequences of testing, previous knowledge about BRCA testing), risk perception (anticipatory worry about developing cancer, feelings of risk), and childbearing intentions. Except for the latter, higher levels of these predictors were associated with a higher likelihood to choose surgery.

**Conclusion:** Our findings shed light on factors associated with greater intention to undergo prophylactic oophorectomy, providing useful insights for genetic counselling.

**EMP1.09****Response to Positive Cancer Genetic Testing Results -The Cyprus Experience.***E. Spanou Aristidou<sup>1</sup>, T. Delikurt<sup>1</sup>, G. Kallikas<sup>2</sup>, G. Tanteles<sup>1</sup>, A. Hadjisavvas<sup>3</sup>, M. Loizidou<sup>3</sup>, K. Kyriacou<sup>3</sup>, V. Christophidou Anastasiadou<sup>1,4</sup>;**<sup>1</sup>Clinical Genetics Clinic, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>2</sup>The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>3</sup>Department of Electron Microscopy, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, <sup>4</sup>Clinical Genetics Clinic, Archbishop Makarios Medical Centre, Nicosia, Cyprus.*

Social parameters, family dynamics and the general appreciation for genetic testing by wider society vary from one culture to the next. It is therefore important to consider these factors within a specific culture when hoping to maximize the effective awareness of the appropriate individuals of this culture, regarding their genetic status. In an effort to indeed consider these factors, in our paper we will examine variables such as how the patient responds to a positive genetic status, how effectively this status is communicated to the biological family, and the response of the biological family, as well as where and how misinformation may occur.

We will review the data accumulated through the patients seen at the clinical genetics clinic, with respect to their demographic data, their socioeconomic status and diagnosis. Also we will discuss how these responses have evolved over the last twelve years since the introduction of cancer genetic counselling and testing as a service offered in Cyprus. The aim of this study is to draw conclusions that will better aid professionals in their attempt to offer quality services to these families.

**EMP1.10****Exploring clinicians' attitudes about using aspirin for risk reduction in people with Lynch Syndrome (LS) without personal diagnosis of Colorectal Cancer (CRC)***B. Meisen<sup>1</sup>, Y. Chen<sup>1</sup>, M. Peate<sup>1</sup>, T. Wong<sup>1</sup>, J. Kirk<sup>2</sup>, R. Ward<sup>1</sup>, A. Goodwin<sup>3</sup>, J. Hiller<sup>4</sup>, A. Trainer<sup>5</sup>, F. McCrae<sup>6</sup>, G. Mitchell<sup>7</sup>;**<sup>1</sup>Prince of Wales Clinical School, University of New South Wales, Randwick, Australia, <sup>2</sup>Westmead Hospital, Westmead, Australia, <sup>3</sup>Concord Cancer Centre, Concord, Australia, <sup>4</sup>Swinburne University of Technology, Melbourne, Australia, <sup>5</sup>Peter MacCallum Cancer Centre Melbourne, Melbourne, Australia, <sup>6</sup>Royal Melbourne Hospital, Melbourne, Australia, <sup>7</sup>BC Cancer Agency, Vancouver, BC, Canada.*

**Background:** Recent research has shown that aspirin reduces the risk of Lynch syndrome (LS)-associated cancers. Little is known about clinicians' attitudes, current practice and perceived barriers to recommending aspirin as a RRM.

**Aim:** To explore the attitudes of Australian clinicians towards using aspirin as a RRM.

**Methods:** Clinicians were invited to complete an online survey. Topics included their LS clinical experience, views and practice of recommending aspirin as a RRM, and knowledge about clinical risk management guidelines for LS. Comparison of attitudes between the professional groups: familial cancer clinic (FCC) staff vs non-FCC staff, genetics professionals vs non genetics

professionals: gastroenterologist and colorectal surgeons) was performed. Results: Data analysis is ongoing. 155 respondents: 8 medical oncologists, 7 clinical geneticists, 26 genetic counsellors, 52 colorectal surgeons, 44 gastroenterologists, 7 nurses and 11 other health professionals completed the survey. Significantly more FCC staff (85%) think that aspirin is an effective RRM, are confident about their knowledge of literature and had discussed aspirin's potential role but non FCC staff (55.1%) are more likely to recommend aspirin. Similarly, non-genetics professionals (88% of gastroenterologists and 76.2% of colorectal surgeons) are more likely to recommend aspirin despite genetics professionals (78%) reporting greater confidence in knowledge about the literature.

Discussion: FCC's staff's incongruent attitude between perception of aspirin's efficacy and actual practice of recommending aspirin warrants further research to understand the underlying reasons. Non FCC staff and non-genetics professionals' higher likelihood of recommending aspirin despite reporting lower confidence suggest possible need for training programs.

#### EMP1.11

#### The efficacy of psychosocial interventions for familial colorectal cancer: a systematic review

*A. M. Ciucă, R. Moldovan, A. Băban;  
Babeş-Bolyai University, Cluj-Napoca, Romania.*

Introduction: Psychosocial interventions are often recommended for individuals with a personal or familial history of colorectal cancer but their impact on various outcomes has been less explored. Our objective was to investigate the impact of psychosocial interventions on cognitive (knowledge about cancer genetics, perception of risk), affective (distress, anxiety, depression) and behavioural (uptake of genetic testing, uptake of screening and surveillance) outcomes in patients diagnosed with colorectal cancer or family members at risk.

Material and Methods: An extensive search was conducted in electronic databases (PubMed, PsycInfo and Cochrane) investigating the literature published until January 2016. We included studies which investigated the efficacy of psychosocial interventions for colorectal cancer; clearly defined the psychosocial interventions; included patients diagnosed with colorectal cancer or family members at risk. Two authors independently assessed the quality of studies.

Results: Studies included in this analysis mainly explored Hereditary Non-polyposis Colorectal Cancer, Familial Adenomatous Polyposis and other familial colorectal cancers without an established genetic factor. The interventions investigated in the studies that met our inclusion criteria are genetic counselling, educational sessions and psychological interventions. Our review shows that psychosocial interventions are efficient in terms of several cognitive, affective and behavioural outcomes.

Conclusions: A number of psychosocial interventions are aimed at improving affective, cognitive and behavioural outcomes in individuals affected or at risk for familial colorectal cancer and the vast majority of them are efficient. Implications of this study are discussed in detail.

#### EMP1.12

#### Developing the Italian version of the Psychosocial Aspects of Hereditary Cancer questionnaire (I-PAHC)

*M. Franiuk<sup>1</sup>, L. Battistuzzi<sup>2</sup>, E. Molinari<sup>2</sup>, E. Razzaboni<sup>4</sup>, D. Turchetti<sup>5</sup>, P. Tassone<sup>6</sup>, C. Chiorri<sup>7</sup>, L. Varesco<sup>1</sup>;*

<sup>1</sup>IRCCS AOU San Martino-IST, Unit of Hereditary Cancer, Genova, Italy,

<sup>2</sup>Department of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy, <sup>3</sup>Department of Educational Sciences, University of Genova Genova, Italy, Genoa, Italy, <sup>4</sup>Modena Cancer Center, Modena, Italy,

<sup>5</sup>Unit of Medical Genetics, Department of Medical and Surgical Sciences (DIMEC), S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy,

<sup>6</sup>Medical Oncology and Translational Medical Oncology Units, Department of Experimental and Clinical Medicine, Magna Graecia University, AOU Materdomini Catanzaro, Campus Salvatore Venuta, Catanzaro, Italy,

<sup>7</sup>Department of Educational Sciences, University of Genova Genova, Italy, Genova, Italy.

Introduction: The Psychosocial Aspects of Hereditary Cancer (PAHC) questionnaire has been recently developed in the Netherlands to assess psychosocial issues associated with cancer genetic counselling (CGC) in counselees. The PAHC includes 27 items organized into six problem domains (genetics, practical issues, family, living with cancer, emotions, and children), and has proven to be useful in improving counsellor-counselee communication and decreasing counselee distress. We aimed at developing an Italian adaptation of the PAHC (I-PAHC), as no such questionnaire is currently available in Italy. Methods: This is a prospective multicentre observational study including three stages: (i) development of the I-PAHC; (ii) pilot study aimed at testing item readability; (iii) validation study.

Results: We present here the results of stages (i) and (ii). During the translation process the research team decided to add two further domains (perceived social support and motivation to undergo genetic testing), and ex-

panded the emotions domain to include positive emotions. The I-PAHC thus comprises 49 items. Thirty participants were administered the I-PAHC and interviewed to investigate item readability. While most of the items were found to be easy to understand and to score, some required revision to improve comprehensibility, and others were deleted as irrelevant or redundant.

Conclusions: The I-PAHC seems likely to afford comprehensive content coverage of psychosocial problems related to cancer genetic counselling, and study participants have thus far reacted positively to the instrument. The next step of the project will be to test the psychometric properties of the questionnaire in a larger sample.

#### EMP1.13

#### The provision of youth friendly genetic counselling

*A. Lewis, K. Thompson, L. Holland, M. Young;  
Peter MacCallum Cancer Centre, East Melbourne, Australia.*

Over the last 20 years the genetic counselling literature has highlighted the struggles experienced by genetic counsellors in working with young people<sup>1</sup>. As the Peter MacCallum Familial Cancer Centre has experienced an increased number of young people (15-26) seeking information about their cancer genetic risk and predictive genetic testing, a collaboration was established with onTrac, the Victorian Adolescent and Young Adult Cancer Service, to develop a youth friendly model of genetic counselling, to overcome some of these challenges.

This evidence based model of genetic counselling, aims to support the unique needs of young people who are in the midst of a developmentally sensitive time, promote their autonomy whilst also acknowledging the needs of their parents.

This presentation will examine the increase in young people attending for cancer genetic counselling, review some of the factors influencing young people attending and discuss a new model of genetic counselling for young people.

<sup>1</sup>Duncan RE, Young MA. Tricky teens: are they really tricky or do genetic health professionals simply require more training in adolescent health? *J.Pers.Med.* 2013;10(6):589-600

#### EMP1.14

#### Contemplating growing older with Cystic Fibrosis(CF): The experiences of adult CF patients taking ivacaftor, a new therapy

*A. B. Watford<sup>1</sup>, M. McAllister<sup>1</sup>, A. Prosser<sup>2</sup>, C. O'Leary<sup>2</sup>, S. K. Phillips<sup>3</sup>, R. I. Ketchell<sup>2</sup>, R. Belk<sup>4</sup>;*

<sup>1</sup>Cardiff University, Cardiff, United Kingdom, <sup>2</sup>Cardiff & Vale University Health Board, All Wales Adult CF Centre, Cardiff, United Kingdom, <sup>3</sup>University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom, <sup>4</sup>University of Manchester, Manchester, United Kingdom.

Introduction: Ivacaftor (Kalydeco™), a CF transmembrane conductance regulator (CFTR) gene potentiator, is a treatment approved for CF patients with at least one p.Gly551Asp CFTR-mutation. It is clinically effective in short-term clinical trials and small real-world settings in CF patients. Ivacaftor's apparent potential to change clinical outcomes highlights the need for research exploring the psychosocial impact of ivacaftor for patients and implications for their care. This service evaluation explored the experiences of a group of patients on ivacaftor within two UK adult CF centres using qualitative methods.

Methods: Participants were recruited from the All Wales Adult Cystic Fibrosis Centre and the Bristol Adult Cystic Fibrosis Centre. Seven adult CF patients participated in semi-structured interviews. Interview transcripts were analysed using interpretative phenomenological analysis.

Results: Participants reported significant improvements in respiratory symptoms and a decrease in their CF treatment regimes since taking ivacaftor. All were tentatively contemplating growing older and new life choices including having children and pursuing a career. Some were experiencing anxiety about the treatment's side-effect of weight gain and reported dissatisfaction with their body image since taking ivacaftor. Ivacaftor experiences were novel for the adult with CF: patient narratives emerged of negotiation of care between the patient and service provider.

Conclusion: The changing perceptions of these patients towards their future health and ageing with CF has potential implications for how CF centres and other NHS services personalise care for this emerging cohort who are considering new challenges such as weight gain, decisions about future careers and having children.

**EMP1.17****Direct-to-consumer personal genome testing: a futile search for security?**

**J. Savard, J. Mooney-Somers, A. Newson, I. Kerridge;**  
*The University of Sydney, Camperdown, Australia.*

Advances in genetics and genomics provide new and powerful ways for individuals to understand their past and predict their futures. These technologies are now available in the marketplace and are heavily and directly promoted to consumers. Although some may see this as an inevitable and valuable democratization and dissemination of biomedical knowledge, it is not unproblematic. Importantly, there is often a discrepancy between consumer expectations of what personal genome tests may provide and the explanatory power that they find they have.

In this paper, I present findings from a mixed methods research project that explored Australian consumers' beliefs, knowledge, expectations and experiences of direct-to-consumer personal genome testing (DTCPGT). This research involved a public survey, in-depth qualitative interviews with consumers of DTCPGT and an autoethnography.

The results of this research reveal that while DTCPGT may have value, both for consumers and for society, this is contextually dependent. Importantly, seeking DTCPGT appears to be best understood as a process of 'securitization.' That is, an individual will seek genetic knowledge about their self through DTCPGT because they believe it will enable them to confirm 'certainties' about their life and to decrease 'uncertainties'. Unfortunately, the reality is that DTCPGT instead often leads to the proliferation of uncertainty. This is because the scientific knowledge is still limited. We also lack knowledge as to what psycho-social impact test results may have. Further, gaining any form of information unavoidably reveals the gaps in that knowledge.

**EMP1.19****Forum OncoGenEtica: providing Italian healthcare professionals and researchers with opportunities to discuss ethical problems that arise in the practice of cancer genetics**

**L. Battistuzzi<sup>1</sup>, P. Ghiorzo<sup>2,3</sup>, S. Sciallero<sup>4</sup>, M. Parker<sup>5,6</sup>, L. Varesco<sup>7,8</sup>,**  
<sup>1</sup>Dept. of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy, <sup>2</sup>Genetics of Rare Cancers, Dept. of Internal Medicine and Medical Specialties, University of Genoa, Genoa, Italy, <sup>3</sup>IRCCS AOU San Martino-IST, Genoa, Italy, <sup>4</sup>Medical Oncology Unit 1, IRCCS AOU San Martino-IST, Genoa, Italy, <sup>5</sup>Ethox Centre, University of Oxford, Oxford, United Kingdom, <sup>6</sup>UK Genetics Club, Oxford, United Kingdom, <sup>7</sup>Unit of Hereditary Cancer, IRCCS AOU San Martino-IST, Genoa, Italy, <sup>8</sup>On behalf of the Working Group on Cancer Genetics of the Italian Society for Human Genetics, Rome, Italy.

**Introduction:** Many decisions that healthcare professionals and researchers working in cancer genetics (HPRCGs) make in their daily practice have a moral dimension, and it is not uncommon for HPRCGs to be faced with situations involving difficult value judgements. Drawing from the experience of the UK Genetics Club, the Forum OncoGenEtica was established in 2014 to provide Italian HPCGs with opportunities to discuss the practical ethical problems that arise in their work.

**Materials and Methods:** The Forum OncoGenEtica meets twice yearly and is open to healthcare professionals and researchers working in cancer genetics or otherwise involved with families affected by hereditary cancer. Participants are encouraged to present ethically problematic cases. Meetings are informal and discussion is facilitated by a bioethicist, a molecular geneticist, an oncologist and a clinical geneticist. After each meeting a report is circulated among participants summarizing the cases and the salient points of the discussion.

**Results:** As of January 2016 the Forum has organized three meetings and a conference at the San Martino-IST Research Hospital in Genoa, Italy. Overall attendance has exceeded 200 participants, mostly from hospitals or universities in northern Italy. Eighteen cases have been discussed, with balancing conflicting interests and managing confidentiality within families being the most frequent topics.

**Conclusions:** The Forum OncoGenEtica has thus far met with a good degree of interest within the Italian cancer genetics community. Two meetings are planned for 2016, one of which possibly at a different location in order to reach HPRCGs from other regions of the country.

**EMP1.20****Development of a scale for assessing attitudes towards cancer genetic testing among Primary Care Providers and Breast Specialists**

**A. Bouhnik<sup>1</sup>, K. N'Diaye<sup>1</sup>, G. Evans<sup>2</sup>, H. Harris<sup>2</sup>, A. Tibben<sup>3</sup>, C. van Asperen<sup>3</sup>, J. Schmidtke<sup>4</sup>, I. Nippert<sup>5</sup>, C. Julian-Reynier<sup>6</sup>,**  
<sup>1</sup>INSERM-U912, Marseille, France, <sup>2</sup>University of Manchester, Manchester, United Kingdom, <sup>3</sup>Leiden University Medical Centre, Leiden, Netherlands, <sup>4</sup>Institute of Human Genetics, Hannover, Germany, <sup>5</sup>Women's Health Research, Münster Medical School, Münster, Germany, <sup>6</sup>Institut Paoli-Calmettes; UMR912, Inserm, AMU, IRD, Marseille, France.

**Objective:** To develop a generic scale for assessing attitudes towards cancer genetic testing (BRCA1/2) and test this scale psychometrically among a

sample of French General Practitioners and Breast Specialists.

**Methods:** A 15 items scale was drawn up in a context of an international project (INCRISC European project), assessing perceived benefits (8 items) and drawbacks (6 items) of the process of breast/ovarian cancer genetic testing (BRCA1/2) as well as an overall indicator of agreement with the fact that expected health benefits of BRCA1/2 testing exceeded negative consequences and justified their medical prescription. These items were included in a self-administered questionnaire mailed to a sample of French doctors: 182 Breast Surgeons (BS), 275 General Practitioners (GPs) and 294 gynaeco-obstetricians. Principal Component Analysis, Cronbach's  $\alpha$  coefficient, and Pearson's correlations were used in the statistical analyses.

**Results:** 4 dimensions emerged from the respondents' responses, and were classified under the headings: "Discrimination-Stigmatisation", "Risk Information", "Prevention-Surveillance", and "Anxiety-Conflicts". Cronbach's  $\alpha$  coefficients on the four dimensions were 0.82, 0.76, 0.62 and 0.57 respectively, and each dimension demonstrated a good correlation with the overall indicator of agreement (criterion validity). Breast surgeons scored lower on the first and fourth dimensions (both negative dimensions) and higher on the third dimension (Prevention-Surveillance). Women doctors scored higher on the fourth dimension.

**Conclusions:** The validation process revealed satisfactory psychometric properties for this scale that could be generic for cancer genetic testing. Confirmatory analysis for the 3 other countries involved in the project is shown in another communication.

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**EMP1.21****Predictive genetic testing in common complex diseases - a Delphi study on a preventive public health genomics model**

**M. B. Hocaoglu<sup>1</sup>, F. Nizam<sup>2</sup>, P. Savas<sup>2</sup>, B. Taner<sup>1,2,3</sup>,**

<sup>1</sup>Eastern Mediterranean University - Faculty of Medicine, Famagusta, Cyprus,

<sup>2</sup>Eastern Mediterranean University - Department of Biological Sciences, Famagusta, Cyprus, <sup>3</sup>Maastricht University - Institute of Public Health Genomics, Maastricht, Netherlands.

**Introduction:** Predictive testing of any kind is of utmost importance for public health. In light of the cost reductions in sequencing technologies, predictive genetic and genomic testing is bound to become an integral part of personalized healthcare as well as public health approaches. Consequently, models that integrate gene and environment interactions are needed to be developed for common complex diseases, as both play a role in disease etiology. This work focuses on a public health genomics model with the aim of improving it via expert evaluation and validation prior to implementation.

**Materials and Methods:** We have previously published a public health genomics model for common complex diseases that integrates genetic testing and environmental factors for prevention of disease onset. In this study, we utilize the widely-used Delphi technique to improve and validate this particular model, and to facilitate discussion on setting associated ethical guidelines. The Delphi technique involves rounds of structured discussions of the model by an expert panel with the aim of building consensus.

**Results:** The expert-improved and validated model for predictive genetic testing in common complex diseases will provide a basis for development and standardization of associated healthcare guidelines and policies. In parallel, this study will provide a platform for discussion and formulation of ethical guidelines and regulations for implementation of the model.

**Conclusions:** The improved, validated model will present a valuable source for development of effective public health implementations and ethical guidelines for predictive testing of common complex diseases including cancers, cardiovascular conditions, diabetes and psychiatric conditions.

**EMP1.22****Genetic counselling in idiopathic autism: parental knowledge and perspectives**

**M. Codina-Solà<sup>1,2,3</sup>, M. del Campo<sup>4,5</sup>, L. Pérez-Jurado<sup>1,2,3</sup>, C. Serra-Juhé<sup>1,2,3</sup>,**

<sup>1</sup>Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain, <sup>2</sup>Neurosciences Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain, <sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Barcelona, Spain, <sup>4</sup>Servicio de Genética, Hospital Universitari Vall d'Hebron, Barcelona, Spain, <sup>5</sup>Division of Dysmorphology and teratology, Department of Pediatrics, University of California, San Diego, CA, United States.

**Introduction:** Autism Spectrum Disorders (ASD) are among the most inheritable neurodevelopmental disorders, but the aetiology remains unknown in the majority of cases. Therefore, most parents must base their reproductive decisions on empiric recurrence risk (RR) estimates. To determine factors influencing family planning and benefits of genetic counselling (GC), we studied knowledge and perceptions in parents with children with idiopathic ASD.

**Materials and Methods:** Parents with at least one child with ASD (n=39) answered a basal questionnaire addressing different topics, such as knowledge, perceived causes and RR or attitude towards genetics. A subset whose child

had obtained negative results in molecular karyotype and exome sequencing (n=15) received GC and a following-up questionnaire afterwards and fifteen days later.

**Results:** Although most parents had received information about ASD, few had seen a medical geneticist or a genetic counsellor. Genetics was the most frequent perceived cause, especially among those with affected relatives. RR was overestimated by most parents and qualitative and quantitative estimates correlated. About half of parents believed that RR affected their family planning and their risk perception was higher compared to those who did not. After GC, quantitative but not qualitative RR estimates lowered and knowledge and favorable opinion towards genetics increased.

**Conclusions:** Although most parents perceived genetic factors as the most common cause of ASD, few had visited a genetics service. Perception of RR affects family planning and is overestimated by most parents. GC can improve knowledge and RR estimates and is key to take informed choices.

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#### EMP1.23

##### Pilot testing of an educational resource on genomic testing for breast cancer risk

**T. Yanes<sup>1</sup>, R. Kaur<sup>1</sup>, B. Meiser<sup>1</sup>, T. Rixon<sup>1</sup>, M. Young<sup>2</sup>, K. Barlow-Stewart<sup>3</sup>, T. Roscioli<sup>4</sup>, G. Mitchell<sup>2</sup>, P. James<sup>2</sup>;**

<sup>1</sup>University of New South Wales, Sydney, Australia, <sup>2</sup>Peter MacCallum Cancer Centre, Melbourne, Australia, <sup>3</sup>University of Sydney, Sydney, Australia, <sup>4</sup>Garvan Institute of Medical Research, Sydney, Australia.

**Introduction:** We are undertaking a prospective, mixed-method study of the psychological and behavioural impact of disclosing genomic testing results for common risk variants associated with breast cancer. An education pamphlet for women considering testing for common risk variants to assess their breast cancer risk was developed and pilot tested. Participants of an existing study: 'Common Genetic Variants and Familial Cancer' were invited to take part in this study.

**Methods:** The two-page education pamphlet provides a brief explanation of rare high-risk gene mutations (BRCA1 and BRCA2) and common risk variants in breast cancer. It covers a range of topics relevant to genomic testing for breast cancer risk, including meaning and implications of results, impact on family members and insurance. The pamphlet was written in accordance with health literacy guidelines and at a grade nine literacy standard (14-15 years old). The pamphlet was pilot-tested with 28 female participants of the parent study. Descriptive statistics were calculated for all responses.

**Results:** All women thought their understanding of the risks and benefits of genetic testing had improved. The majority of participants (68%) reported that the education pamphlet did not make them feel worried, with 71% actually reporting feeling reassured. Participants provided detailed suggestions for amendments including visual presentation, and readability. Feedback provided has since been incorporated and the final pamphlet has been reduced to a grade 8 literacy level (12-14 years old).

**Conclusions:** The revised pamphlet with the information on genomic testing and breast cancer risk will be used in the prospective psychosocial study.

#### EMP1.24

##### How do counselees reach decisions and how can they be adequately supported during decision-making?

**T. Brouwer, H. van Spijker;**

Department of Medical Genetics, Utrecht, Netherlands.

**Introduction:** Much research has been conducted on the outcome of couples' decision-making, when a genetic disorder runs in the family. However, the question of how couples come to decisions, or what information they need to reach an informed choice, has seldom been the subject of research.

**Materials and Methods:** During many years of working with couples, materials have been collected for a new booklet on strategies and supportive action plans for the decision-making process.

**Results:** A new booklet entitled "The desire for children and inherited genetic conditions" (in Dutch: "Kinderwens en erfelijkhed") has emerged from the practice based evidence of supporting couples struggling with reaching decisions whether or how to have children, when one or both parents has a hereditary disease. This booklet describes not only the various reproductive options available to such couples but also various strategies to facilitate their decision making. The booklet also contains tools to assist when the decision-making process threatens to get stuck. One of these tools will be presented on the poster.

**Conclusions:** Written information should not and cannot replace tailored counseling. However, by describing various strategies of decision-making in the field of genetics, written information can be important for both counselees, social workers and genetic counselors. This is particularly so when the genetic counselling entails delicate decisions.

#### EMP1.25

##### Communication of genetic risk information about X-linked disorders: Carrier mothers' perceptions of facilitators and barriers to talking to daughters

**A. Goldman<sup>1,2</sup>, R. MacLeod<sup>1,2</sup>;**

<sup>1</sup>University of Manchester, Manchester, United Kingdom, <sup>2</sup>St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.

**Introduction:** The process of communicating information about Duchenne and Becker muscular dystrophy (DMD or BMD) to girls who may be carriers has been little studied. Mothers are often the primary source of information, both about carrier risk and the nature of the condition, yet mothers' voices are notably absent in the literature. We need to understand how mothers experience this communication process in order to best support them in this task.

**Materials and Methods:** A qualitative study using Interpretative Phenomenological Analysis (IPA) explored how mothers described and reflected on the experience of communicating information about DMD or BMD and carrier risk to their daughters aged <18 years. 6/15 mothers invited to participate through the North West Family Register Service agreed to participate. Semi-structured telephone interviews of around 45 minutes were audiotaped with consent, and transcribed in full for IPA analysis.

**Results:** The six participants described how they had endeavoured to be open and honest with their daughters. Yet, all had struggled with some aspect of the communication process. Notably mothers found it harder to talk about the future, both in terms of the life limiting nature of the condition and their daughters' future reproductive options. We describe the different phases of disclosure reached, and the barriers and facilitators of communication identified.

**Conclusions:** Participants suggested interventions that they felt would aid the disclosure process, notably a greater involvement of Genetic Counsellors at an earlier stage.

#### EMP1.26

##### Developing a decision aid for genomic research participants notified of clinically actionable research findings in the hereditary cancer setting

**A. M. Willis<sup>1</sup>, S. K. Smith<sup>1</sup>, B. Meiser<sup>1</sup>, L. Ballinger<sup>2</sup>, D. M. Thomas<sup>2</sup>, G. Mitchell<sup>3,4</sup>, J. Kirk<sup>5,6</sup>, M. Tattersall<sup>7</sup>, M. Young<sup>3,4</sup>;**

<sup>1</sup>Psychosocial Research Group, Prince of Wales Clinical School, UNSW Australia, Sydney, Australia, <sup>2</sup>The Kinghorn Cancer Centre and Cancer Division, Garvan Institute of Medical Research, Darlinghurst, Australia,

<sup>3</sup>Familial Cancer Centre, Peter MacCallum Cancer Centre, Melbourne, Australia, <sup>4</sup>Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia, <sup>5</sup>Familial Cancer Service, Crown Princess Mary Cancer Centre, Westmead Hospital, Westmead, Australia, <sup>6</sup>Centre for Cancer Research, Westmead Millennium Institute, University of Sydney, Westmead, Australia, <sup>7</sup>Department of Cancer Medicine, Sydney Medical School, Royal Prince Alfred Hospital, Camperdown, Australia.

**Introduction:** Research genomic screening is increasingly used to identify genetic causes of disease, including cancer. However, there is evidence that individuals who are notified of clinically actionable research findings are ill-equipped to make decisions regarding uptake of these findings. While effective decision support for these research participants is clearly required, the broad scope of genomic screening presents unique challenges in resource development. We aimed to develop a resource to fill this decision support gap.

**Methods:** A decision aid booklet was developed based on a systematic review of the literature regarding cancer genetic counselling uptake, the International Patient Decision Aid Standards and the expertise of a steering committee of clinicians, researchers and consumers. The acceptability of the decision aid was assessed by questionnaire among genetic research participants who had previously accessed clinically actionable research findings.

**Results:** All 19 participants stated that the decision aid was easy to read and clearly presented, increased their understanding of the implications of taking up research findings and would be helpful in their decision-making. Seven participants reported low to moderate levels of distress/worry after reading the booklet; however 18 reported some level of reassurance. All participants would recommend the booklet to others considering uptake of clinically actionable research findings.

**Conclusions:** Results indicate the decision aid is acceptable to the target audience and has potential as a useful decision support tool for genomic research participants. The decision aid will be provided to Australian research participants notified of clinically actionable research findings by the International Sarcoma Kindred Study.

## EMP1.27

### Professionals' views and experiences of facilitating family communication about genetics: Findings from a qualitative study

**K. Forrest Keenan, L. McKee, Z. Miedzybrodzka;**

*University of Aberdeen, Aberdeen, United Kingdom.*

**Introduction:** UK guidelines advise genetics professionals to support and encourage family communication about genetic risk. We sought to explore genetics professionals' experiences in clinical practice and to identify effective elements of communication in this area.

**Methods:** In depth interviews with 23 genetics professionals across Scotland, UK.

**Results:** Whilst all participants felt that facilitating family communication about genetics was an important aspect of their role, one third reported giving limited advice and lacked confidence in this area, particularly parent/child communication. Nevertheless, we identified several 'successful' strategies and frameworks which genetics professionals draw upon, including: (1) Initiating a discussion about how/when to tell; (2) Offering to tell children directly (with or without parent present); (3) Offering access to a quick follow-up appointment e.g. after parental disclosure and (4) Accepting ambiguity as there maybe "no clear cut answers." In cases of nondisclosure professionals may: (1) "Chip away" (over months or years); (2) Undertake "emotion work" before involve children or (3) Use other relatives as "different routes" to disclosure e.g. siblings or aunts/uncles. Participants' expressed a need for child/young people focussed counselling aids, including disease specific disclosure guidance, and the time to undertake this work.

**Conclusion:** Genetics professionals use multiple strategies to facilitate family communication about genetics, particularly psychoeducational guidance to parents. However, this remains a challenging and sensitive area in which genetics professionals express a need for further training, resources and evidence based interventions.

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## EMP1.28

### Genetic and social complexities of Non-Syndromic Hearing Loss: the importance of genetic counseling

**D. Salinas, M. Borregán, L. Martínez, A. Cueto, T. Vendrell, M. Del Campo, E. Tizzano;**

*Department of Clinical and Molecular Genetics and Rare Disease Unit, Hospital Vall d'Hebron, Barcelona, Spain.*

**Introduction:** Non-Syndromic Hearing Loss (NSHL) has always created a growing interest due to its genetic heterogeneity and complex social background. We present the first Spanish study that gathers together genetic and social complexities of NSHL, highlighting the importance of genetic counseling. The aims of the study were to evaluate differences in opinions, knowledge and information needs or genetic counseling among the deaf individuals and their families. Knowledge and satisfaction of those who had received genetic counseling were also analyzed.

**Methods:** Participants (n=50) were recruited from different centres and associations from Catalonia (Spain). An ethically validated questionnaire was distributed to the families; data was statistically analyzed with SPSS (v.20)

**Results:** Differences in terms of knowledge, interest in diagnostic test and opinions, defined by the level of immersion in the deaf community and hearing status of partners and relatives were found. Most of the participants (62%) were interested in genetic testing. Feelings towards genetic testing were mostly positive in comparison with previous studies in other populations. We also have seen that testing may not influence significantly on reproductive options although 33% would opt for a reproductive alternative (being PD the first option, 61%). A great increase of knowledge was evidenced between the pre- and post counseling session (37% of correct answers to 71%). The genetic counseling satisfaction score was 28.18 (out of 30).

**Conclusions:** This work reveals an evident need of information about genetics of hearing loss and shows a great increase of knowledge and satisfaction after receiving genetic counseling.

## EMP1.29

### The making, implementation and evaluation of a BRCA information web site for breast - and ovarian cancer patients

**E. Sigler<sup>1</sup>, H. Høberg-Vetti<sup>1,2</sup>, M. Tveit-Haavind<sup>2</sup>, C. Bjorvatn<sup>2,3,4</sup>,**

<sup>1</sup>*Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway, <sup>2</sup>Western Norway Familial Cancer Center, Haukeland University Hospital, Bergen, Norway, <sup>3</sup>Learning and Mastery Center in Bergen, Bergen, Norway, <sup>4</sup>Department of Clinical Science, University of Bergen, Bergen, Norway.*

**Introduction:** As genetic testing of cancer patients increasingly will be part of the diagnostic process, due to treatment consequences, it is important to ensure that the patients receive accurate genetic information within the clinical setting. New methods to provide patients with genetic information

and counseling in the decision process are needed. Through the DNA-BONus study unselected breast-and ovarian cancer patients were offered BRCA1/2 testing and familial cancer risk assessment without prior genetic counseling. We developed and evaluated a web based patient oriented information site, and measured the efficacy of an online decisional aid in genetic testing.

**Material and methods:** From April 2014 to April 2015, eligible patients were consecutively randomized into two groups, intervention group (N= 85) and control group (N= 109). They all received the same information prior to genetic testing, but the intervention group was also provided with a patient oriented web site (log in required) about hereditary breast- and ovarian cancer. The web site was built up using the Interactive Health Communication System model (IUCHS). IUCHS ensures quality assured information and increases the patient's feeling of control and contributes to involve partner in the decisional process.

Different psycho-social measures have been included in the DNA-BONus study. In the present RCT we included two outcome questionnaires; "Openness to discuss cancer in the nuclear family" and "Decisional Conflict Scale".

**Conclusion:** Updated results will be presented, including the use and evaluation of the web site, and the efficacy of the online decisional aid.

## EMP1.30

### How do genetic counsellors give and receive feedback in the peer experiential and reciprocal supervision (PEERS) model?

**H. M. Brandon<sup>1,2,3</sup>, L. Stirling<sup>4</sup>, S. Wake<sup>1,2,5</sup>, M. Kentwell<sup>6,7</sup>,**

<sup>1</sup>*Department of Paediatrics, Faculty of Medicine, Dentistry & Health Sciences, University of Melbourne, Melbourne, Australia, <sup>2</sup>Murdoch Childrens Research Institute, Melbourne, Australia, <sup>3</sup>South West Thames Regional Genetics Service, St George's University Hospitals, London, United Kingdom, <sup>4</sup>School of Languages & Linguistics, Faculty of Arts, University of Melbourne, Melbourne, Australia, <sup>5</sup>Victorian Clinical Genetics Service, Melbourne, Australia,*

<sup>6</sup>*Department of Genetic Medicine and Familial Cancer, Melbourne Health, Melbourne, Australia, <sup>7</sup>Department of Oncology, The Women's, Melbourne, Australia.*

**Introduction:** The Peer Experiential and Reciprocal Supervision (PEERS) model is a unique supervision model developed for genetic counsellors by a single Victorian clinical genetics service. The aim of this study was to undertake further detailed analysis of PEERS model feedback session interactions to inform further development of the model, with a focus on the verbal giving and receiving of feedback. Feedback was explored for initial pair interactions in the PEERS model.

**Methods:** Audio recorded feedback sessions, from 2013 and 2014, for six genetic counsellors were transcribed and reviewed using discourse analysis. Five of the six participants also partook in a focus group to discuss the results with the discussion evaluated using content analysis. The results and conclusions, being derived from this small sample size, may not be considered statistically significant yet serve to provide preliminary information to inform further research.

**Results:** The giving and receiving of feedback was verbalised with explanations for the feedback and utilised co-occurring discourse characteristics of disfluency, hedging and epistemic markers, the latter to state that the assessment was their opinion.

**Conclusions:** It was concluded that feedback interactions were influenced by a participant's culture of professional practice, personal preferences and the PEERS protocol, differing from casual conversation. The manner in which feedback is given and received is to help ensure it is presented in a way which considers the needs of the listener. The findings of this study can be used to enhance participants experience in the PEERS model and provide insights to interprofessional communication.

## EMP1.31

### Prophylactic Mastectomy in Women Carriers of Pathogenic Mutation in Genes BRCA1/2. Has the Usage Increased as an Option for Breast Cancer Management?

**M. Salinas<sup>1</sup>, S. Iglesias<sup>1</sup>, E. Grau<sup>2</sup>, E. Darder<sup>3</sup>, A. Velasco<sup>3</sup>, A. Solanes<sup>2</sup>, A. López<sup>3</sup>, A. Teulé<sup>1</sup>, M. Navarro<sup>2</sup>, G. Capellà<sup>1</sup>, C. Lázaro<sup>4</sup>, A. Izquierdo<sup>3</sup>, J. Brunet<sup>3,1,2</sup>,**

<sup>1</sup>*Heredity Cancer Program, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain, <sup>2</sup>Heredity Cancer Program, Catalan Institute of Oncology, Badalona, Spain, <sup>3</sup>Heredity Cancer Program, Catalan Institute of Oncology, Girona, Spain.*

**INTRODUCTION:** Risks to develop breast cancer in women carrying mutations in BRCA1/2 range between 55 and 86%.

Prophylactic mastectomy (PM) is one of the actions raised by patients to reduce the risk significantly. Until recently, population opted for a clinical follow-up and discarded to use this technique. The mixture of different circumstances, including Angelina Jolie public declaration in February 2013, have produced a notable increase for this type of surgery demand, in both - healthy and breast cancer affected women.

**METHODOLOGY:** In total, 1020 women carriers of mutation have been analyzed and visited in the 3 genetic counselling units of "Institut Català d'Oncologia" during 1998-2006 (618 breast/ovarian or breast and ovarian cancer affected women and 402 healthy women).

**RESULTS:** Until 2013, 707 patients were visited. Among the 235 healthy ones, only 4.6% performed the PM, while among the 472 carriers affected, 14.7% opted for the PM surgery.

Heretofore, the total number of carriers has risen to 1020. Overall, 10.94% of the healthy patients and 18.9% of the affected ones have chosen the PM. In percentage terms in 2013-2016 period, PM have doubled compared the over previous 14 years.

**CONCLUSION:** The so call "Angelina Jolie's Effect" has influenced on the decisions of our patients: a considerable increase of PM among healthy patients has been noticed while it has remained quite stable among the affected ones.

### EMP1.32

#### **A qualitative study exploring the impact of next generation sequencing panel testing for individuals with retinal dystrophy**

**H. G. Jones<sup>1</sup>, E. V. McVeigh<sup>2</sup>, G. Hall<sup>3</sup>, G. Black<sup>3</sup>;**

<sup>1</sup>Nottingham University Hospitals, Nottingham, United Kingdom,

<sup>2</sup>Birmingham Women's Hospital Foundation Trust, Birmingham, United Kingdom, <sup>3</sup>Central Manchester Foundation Trust, Manchester, United Kingdom.

**Background:** Inherited retinal dystrophies (IRD) are a highly genetically heterogeneous disease group causing progressive vision loss. Diagnostic testing for IRD involves Next Generation Sequencing (NGS) of a 176 IRD gene panel. NGS only identifies pathogenic variants in IRD genes in 50% of cases. Many patients remain undiagnosed. For a fraction of the patients, NGS testing produces incidental findings (IF), including variants of uncertain significance (VUS) and carrier status results. This three-armed study aimed to define the impact NGS panel testing has on patients.

**Materials and Methods:** Semi-structured qualitative telephone interviews investigated patient views of NGS panel testing and the impact of results. 23 participants were recruited from the Manchester Centre for Genomic Medicine. 5 participants received a pathogenic, positive result, 11 received an inconclusive, negative result and 7 received an IF result. Interviews were transcribed verbatim and analysed using Interpretive Phenomenological Analysis (IPA).

**Results:** Collectively, 5 key themes were identified: The Retinal Dystrophy Journey; Information from Health Care Services; Shared Impact; Uncertain Future; Disease-Specific Impact of NGS Testing.

**Conclusion:** Participants receiving positive or IF results value the information and have a good understanding of possible implications. Disclosure of IF had no adverse implications in this patient cohort. In contrast, participants with negative or inconclusive results were left disappointed by genetic testing. The importance of consent, the value of full discussion around results, even if uncertain, is highlighted. Clinicians need to be aware of the challenges any NGS testing results can bring and offer continual support throughout follow-up

### EMP1.33

#### **"There is a chance for me" - Interactional analysis of risk communication in Advanced Maternal Age (AMA) genetic counselling sessions in South Africa**

**T. Wessels<sup>1</sup>, C. Penn<sup>2</sup>;**

<sup>1</sup>Division Human Genetics, Department of Pathology, University of Cape Town, Cape Town, South Africa, <sup>2</sup>Health Communication Research Unit, University of the Witwatersrand, Johannesburg, South Africa.

Providing risk information is central to genetic counselling. Many studies have examined risk communication, but the focus has been on professional and patient perspectives. Less information is available on risk communication in interactions. This study aimed to examine genetic counsellors' (GCs) risk communication in multicultural genetic counselling sessions with women of advanced maternal age (AMA).

Six GCs (2 to 20 years experience) conducted 17 AMA sessions in English (women's second language). The sessions were video and voice recorded and transcribed verbatim. Data were analysed using conversation analysis (CA). CA examines discourse as a topic, i.e. describing the turns, its functions and how these functions are accomplished.

Analysis revealed that the GCs presented the risk of having a baby with a chromosome abnormality and the risk of spontaneous abortion due to the procedure in a number of ways. They presented the risks as odds, percentages, positives, negatives and used analogies. Examination drawing on CA principles revealed that both counsellors and women orientated to an interpretation of the risk. The women's focus was on the meaning for her baby while the GC's focus was on numerical understanding.

The research showed the power of CA methodology to gain new insights into old problems. Importantly, the study revealed a mismatch between the

women and the GCs agendas related to the implications of the risks. This mismatch can result in missed opportunities in exploring patients' emotions, decisions and the implications of their choices.

### EMP1.34

#### **Adaptation in families of individuals with Down syndrome from 8 countries**

**M. L. Van Riper, G. Knafl;**

*University of North Carolina Chapel Hill, Chapel Hill, NC, United States.*

**Introduction:** Prenatal testing for Down syndrome has become a routine part of prenatal care in many countries. Unfortunately, many expectant families find it difficult to make well-informed decisions about positive tests results because they receive limited information about life with Down syndrome or the information they receive is outdated and inaccurate. The main purpose of this study was to examine family functioning in families of individuals with Down syndrome from Brazil, Ireland, Korea, Portugal, Spain, Thailand, United Kingdom and USA.

**Materials and Methods:** 1358 parents completed a survey designed to assess family functioning and key dimensions of the Resiliency Model of Stress, Adjustment and Adaptation.

**Results:** Mean family functioning for the overall sample, as well for 6 of the 8 countries fell within the average range for family functioning. It fell within the increasing strengths range for the USA and the increasing problems range for Thailand. Family functioning got worse with increasing child's age. Family functioning was significantly better with greater ability, hardness, mutuality when partnered, and affirmative communication and significantly worse with greater strains and incendiary communication. Over 46% of the parents viewed the fact that their child had Down syndrome as "a blessing in disguise."

**Conclusions:** Findings contribute to our understanding of family functioning in families of individuals with Down syndrome, as well how culture and family factors interact and shape how families respond.

### EMP1.35

#### **Treatment of hemophilia - what do mothers say?**

**C. von der Lippe<sup>1</sup>, J. Frich<sup>2</sup>, A. Harris<sup>3</sup>, K. N. Solbrække<sup>2</sup>;**

<sup>1</sup>Centre for Rare Disorders, Oslo University Hospital, Oslo, Norway, <sup>2</sup>Institute of Health and Society, University of Oslo, Oslo, Norway, <sup>3</sup>Dep. of Technology and Society Studies, Maastricht University, Maastricht, Netherlands.

**Introduction:** In Norway most boys with hemophilia start with prophylactic treatment around 18-24 months of age. Health professionals administer treatment initially, and parents usually provide treatment from when the child is around four years. Caring for a child with hemophilia may be demanding and stressful. Qualitative research on how parents, and especially carrier mothers, experience the treatment of their sons with hemophilia is limited. The aim of the study is to offer qualitative insights about mothers' experiences about treating hemophilia in different settings.

**Materials and Methods:** We have conducted a qualitative study to explore women's experiences of being a carrier of hemophilia and mother to a boy with hemophilia. We collected data through in-depth interviews with 16 women who were carriers and mothers of a child with hemophilia. We analyzed the data using an inductive thematic analytical approach.

**Results:** The mothers were grateful for the availability of treatment. Hospital treatment was perceived as challenging from a practical perspective. Home treatment was preferred when the boys grew older, but mothers experienced both practical and emotional challenges related to administration of treatment. Repeated venipuncture was especially difficult. The lack of competence about hemophilia displayed by health professionals was frustrating and stressful.

**Conclusion:** More in-depth knowledge of the experiences of mothers of boys with hemophilia, especially regarding treatment, could help health professionals better understand parents' and children's situation, and may facilitate tailored management and support.

### EMP1.36

#### **A qualitative study to explore quality of life in Brazilian families with children who have intellectual disability**

**D. G. Melo, V. A. Santos, R. V. V. Tomaz, S. A. Rodrigues, L. R. S. de Avó, C. M. R. Germano;**

*Department of Medicine, Federal University of São Carlos, São Carlos, São Paulo, Brazil.*

**Introduction:** It is estimated that 1.4% of the Brazilian population has some degree of intellectual disability. Families with a member with intellectual disability are subject to additional stressors that can affect their quality of life. This study aimed to examine family quality of life among Brazilian mothers with children who have intellectual disability.

**Methods:** Individual semi-structured interviews were conducted with 30 mothers, selected by convenience. The interviews were then transcript, encoded and analyzed using categorical thematic analysis technique, considering nine precategories: family health, financial well-being, family relationships, support from others people, support from services, family values, career, recreation, and community interaction. Themes were examined in order to give a comprehensive and interpretative approach to the results.

**Results:** The interviews revealed that the care of disabled child is centered on the mother, who usually stops her professional career or studies, changing the family relationships. The religious coping appeared as a common strategy adjustment and many mothers believed having a disabled child as part of one's destination. The disabled children had less access to services and support than they needed in health, education and recreation. Family quality of life was also negatively affected by financial restrictions and difficulties in social interactions.

**Conclusions:** Emotional and psychological support for all family members, as well as social and practical support comprising income distribution and access to appropriate care, proved to be essential for the welfare of the disabled person and his family.

This survey was supported by the São Paulo Research Foundation (FAPESP, grant 13/24498-8).

## EMP1.37

### Evaluation of genetic counselling in clinical practice: using patient-reported outcome measures for continuous quality improvement

*M. McAllister<sup>1</sup>, A. McDermott<sup>2</sup>, D. Ferrick<sup>1</sup>, C. Thomas<sup>1</sup>, A. Costal Tirado<sup>3</sup>, J. Harris<sup>4</sup>, A. Edwards<sup>4</sup>;*

<sup>1</sup>*Cardiff University, Cardiff, United Kingdom, <sup>2</sup>Cardiff Business School, Cardiff, United Kingdom, <sup>3</sup>University of Barcelona, Barcelona, Spain, <sup>4</sup>Cardiff & Vale University Health Board, Cardiff, United Kingdom.*

The aim in this study was to explore views of genetics clinicians in Wales about usefulness and feasibility of using patient-reported outcome measure (PROM) data for continuous quality improvement in genetic counselling. PROMs data were collected using mailed self-completion questionnaires between February-July 2015. PROMs used were Genetic Counselling Outcome Scale (GCOS-24) and the generic Euroqol (EQ-5D) before and after clinic attendance, and a post-clinic audit tool. EQ-5D is the generic PROM preferred by the UK National Institute for Health & Clinical Excellence, the national agency charged with promoting clinical excellence in the UK National Health Service (NHS). Qualitative interviews with eight participating clinicians explored their perceptions following monthly team meetings to monitor progress. Paired before-after PROMs data were collected from 96 patients (response rate: 23% pre-clinic, 45% post-clinic). Quantitative data analysis demonstrated statistically significant improvement in patients' GCOS-24 scores after clinic attendance ( $p<.001$ ), and high post-clinic scores on the audit tool but no significant change in EQ-5D scores. Monthly meetings with clinicians increasingly focused on change scores on individual GCOS-24 items and strategies the clinical team could adopt to improve these change scores over time. Qualitative analysis of interview transcripts demonstrated that participating clinicians valued use of PROMs data to inform continuous quality improvement initiatives using Plan-Do-Study-Act cycles, the NHS preferred quality improvement approach. Participating clinicians considered the clinical genetics-specific GCOS-24 the most appropriate PROM. Findings were used as a basis for discussions about quality improvement actions for the genetic counselling team.

## EMP1.38

### Experiences of women who had a carrier test for Becker muscular dystrophy as a teenager

*R. Redmond<sup>1,2</sup>, D. Scotcher<sup>1,2</sup>;*

<sup>1</sup>*University of Manchester, Manchester, United Kingdom, <sup>2</sup>Saint Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.*

Despite guidelines it remains unclear when it is appropriate for a minor to have genetic testing. This study explores the experiences of women who had a carrier test for Becker muscular dystrophy (BMD) as a teenager. Three research questions were investigated:

- what motivates women under 18 to request carrier testing?
- what is the impact on a young woman of having a carrier test?
- what do women think about the support and counselling they received?

Seven women were recruited from the Manchester Family Register Service who had a carrier test for BMD as a teenager. Semi-structured, qualitative telephone interviews were carried out and themes were exposed using interpretative phenomenological analysis.

The results show that the teenagers were often motivated to have a genetic test by their parents or by living and growing up with the condition, and that the majority of the participants were upset with their result but with time managed to adjust well. Some participants said that they did not fully understand the implications of their test result at the time. In general, women were satisfied with the level of genetic counselling received.

Genetic counsellors should consider a number of implications for future practice. (1) The information given should be tailored to each teenager, (2) the teenager should be made aware that prenatal options are available, (3) conversations surrounding how and when to discuss carrier status with a potential partner should be covered, (4) and follow-up should be negotiated with teenagers who are carriers and who are non-carriers.

## EMP1.39

### Psychological Support in Cancer Genetics: a review of Current Practice

*C. Firth, V. Tripathi, S. Rose, C. Compton, E. Haque; Guys and St Thomas' NHS Trust, London, United Kingdom.*

Genetic testing for hereditary cancer conditions has many psychosocial impacts on, e.g., general emotional well-being, sense of identity and body image. The Guys' cancer genetics service offers dedicated clinical psychology input for patients at increased familial risk, such as BRCA1 and BRCA2 mutation carriers through the BRCA one-stop MDT clinic, risk reducing surgery pathways and BRCA support groups.

**Objectives:** The survey investigates the psychosocial needs of patients, their satisfaction with available psychosocial support and the acceptability and utility of psychological screening tools in the BRCA one-stop MDT clinic.

**Methods:** Over a five month period patients were asked to complete an online survey asking uptake of and satisfaction with psychosocial support received. In addition a sample of 30 patients attending the BRCA clinic were asked their views about using psycho-social screening tools including the BRCA self concept scale (Esplen et al, 2009).

**Results:** Preliminary data shows high satisfaction with psychosocial services in the cancer genetics service. It also shows the usefulness of psychological screening tools which are tailored to those at increased familial cancer risk.

**Conclusion:** So far the cancer genetics service has used the Distress thermometer and the BRCA Self concept scale. We are conducting a study to investigate the acceptability and usefulness of these tools in our BRCA one-stop multidisciplinary clinic. Preliminary data suggests that these tools are important for understanding which patients may be vulnerable after BRCA testing and which might require additional support. Recommendations for future use of these tools will be discussed.

## EMP1.40

### Reproductive decision making: Interviews with mothers of children with undiagnosed developmental delay

*E. L. Pond<sup>1</sup>, R. Dimond<sup>2</sup>;*

<sup>1</sup>*Wessex Clinical Genetics Service, Southampton, United Kingdom, <sup>2</sup>School of Social Sciences, Cardiff University, Cardiff, United Kingdom.*

**Introduction:** This qualitative study explores the experiences of parents who have a child with an undiagnosed developmental disorder; focusing particularly on reproductive decisions. The research aim was to explore the reproductive decision-making process and examine the contributing factors that influence these decisions, the reasoning behind the decisions made and the emotional consequences.

**Methods:** Data was collected from in-depth semi-structured interviews with five mothers of children without a diagnosis. Transcripts were analysed using interpretative phenomenological analysis.

**Results:** Two broad themes of living without a diagnosis and the impact on reproductive decisions were identified. Participants spoke of needing a diagnosis as an explanation for their child's condition; without which they struggled with the uncertainty of their child's future. Perceptions of risk, the potential impact a child would have on their current children, expectations of a family and the desire for another child, were factors that impacted reproductive decisions.

**Conclusions:** Reproductive decision making for parents of a child without diagnosis is a complex and emotional task that involves weighing up both practical and emotional factors. A lack of control was an overarching issue expressed by participants, who felt that they had no control over diagnosis and an unknown future ahead. This lack of control extended to the reproductive decisions of some participants, who felt the decision had been removed from them, leaving them unable to fulfil their desires to have another child. Other participants found alternative mechanisms to manage this issue, in order to regain some sense of control or relinquish control fully.

## EMP1.41

### The experiences of women who have had carrier testing for Duchenne muscular dystrophy under the age of 18

*H. Fraser<sup>1,2</sup>, D. Scotcher<sup>1,2</sup>;*

<sup>1</sup>*University of Manchester, Manchester, United Kingdom, <sup>2</sup>Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom.*

Though carrier testing of adolescent females for Duchenne muscular dystrophy (DMD) remains an area for discussion due to ethical questions over autonomy, it is still widely practiced. The gap that exists between guidelines - some of which are arguably outdated - and practice is something that requires addressing.

Through the use of purposive, semi-structured interviews, this study endeavoured to investigate what had motivated a group of women to seek carrier testing under the age of 18, and the subsequent impact of test results. Data was analysed using Interpretative Phenomenological Analysis.

Although parents were reported as having had an integral role throughout the testing process, all participants believed they had been in control of the decision to have carrier testing. Women stated that they had felt they had needed to know their carrier status during adolescence, largely as a means to alleviate uncertainty. There was on the whole a minimal negative reaction to testing. Although reproductive factors were framed as having been a key motivator prior to testing, test results appear to have had little impact on subsequent reproductive decisions. This discrepancy creates a *pregnancy paradox*. A number of women reported having not had the opportunity for genetic counselling, and having received little psychosocial input.

This study is indicative that carrier testing for DMD during adolescence does not have a lasting negative impact, and that adolescent girls feel autonomous in their decision to undergo testing. The reported lack of prior psychosocial discussion is, however, something that would benefit from further research.

#### EMP1.42

#### The education pathways in clinical genetics - insight of resident

**A. Pietrzkyk;**

*Genetic Clinic/Cytogenetic Unit, Pomeranian Medical University, Szczecin, Poland.*

Clinical genetics is a relatively new branch of medicine in which physicians can choose to specialize.

The available residency programs vary among different countries but usually they consist of training in genetic clinic, rotations in diverse hospital departments and outpatient clinics and laboratory practice in cytogenetics, biochemical and molecular genetics. Nearly all of them are run by academic medical centres thus strong emphasis is put on research opportunities and education services. The number of physicians trained, still in training or even considering clinical genetics remains too low what is mostly connected to distinctiveness of specialty as well as economic reasons.

As the field of clinical and laboratory genetics is developing rapidly, advancing both the field of diagnostic tools and therapeutic measures the need for clinical geneticists is constantly growing, outnumbering the resources. Thus the accessibility of genetic service might be limited in smaller facilities and availability of consultations within other departments.

I have spent 3,5 year of residency sharing my time between various hospital department, training in genetic clinic and laboratory practice on regular basis. This balanced system has given me an enormous opportunity to create and work within interdisciplinary teams, provide genetic counseling to the groups of patients that would not be reached, quicken the right diagnosis and arrange better management and care. The benefits are shown through actual patients' reports and compared to other education systems.

#### EMP1.43

#### An evaluation of The Genetic Support Network of Victoria: A mixed methods study based on stakeholder participation

**L. A. Di Pietro<sup>1,2,3</sup>,**

<sup>1</sup>*Murdoch Childrens Research Institute, Parkville, Australia, <sup>2</sup>Genetic Support Network of Victoria, Melbourne, Austria, <sup>3</sup>Victorian Clinical Genetics Services, Melbourne, Australia.*

**Introduction:** Individuals affected by genetic and rare conditions have unique support and health care needs. A necessary prerequisite for providing appropriate and accessible supportive services is to understand these needs within a rapidly developing genetics health environment. This study aims to determine the information and support needs of individuals who had previously accessed the Genetics Support Network of Victoria (GSNV) and to explore their beliefs and attitudes toward the network. Taking a participatory evaluation approach, the views of stakeholders and GSNV staff members were additionally explored to uncover avenues for organisational improvement.

**Materials/Methods:** Participants were GSNV service users who had accessed the service within the past 12-months, (genetic/rare disease community, genetic health professionals, researchers and support group leaders), representatives from key stakeholder groups and GSNV staff members (n=40). Five focus groups were conducted with participants grouped together based on the aforementioned categories. Adopting a phenomenological approach, the focus group discussions were transcribed verbatim and coded using thematic analysis to identify major themes.

**Results:** Preliminary data will be presented revealing important themes identified across the group discussions related to participants' understanding of the GSNV's role, beliefs and attitudes, unmet information and support needs and ideas for organisational growth and change.

**Conclusion:** This research will provide important insight into the experiences of members of the genetics health community and their information/ support needs. Participant perspectives will be integrated to provide an overall model

of a national genetics support service that is accessible, maintains feasibility and is grounded within service users' experiences.  
louisa.dipietro@vcgs.org.au

#### EMP1.44

#### Swedish healthcare providers' perceptions of preconception genetic carrier screening (PCS)- a qualitative study

**A. Matar, U. Kihlbom, A. T. Höglund;**

*Center for Research Ethics and Bioethics, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden.*

**Introduction:** preconception genetic carrier screening (PCS) is a new approach to screen couples in the general population, without priori risk and planning pregnancy, for autosomal recessive traits. A couple would be screened for many conditions via expanded screening panels at one go. This technique is currently being piloted in the Netherlands but has not been implemented in Sweden.

**Materials and Methods:** the study explores perceptions of PCS via expanded panels among Swedish healthcare providers, with focus on ethical aspects. Eleven healthcare professionals, including clinicians, geneticists, a midwife and a genetic counselor, from academic and clinical institutions in mid-Sweden were interviewed in depth, using a semi-structured questionnaire. Interviews were recorded, transcribed verbatim and content analyzed for categories and subcategories.

**Results:** participants expressed ethical concerns regarding discrimination, medicalization, prioritization of healthcare resources and effects on reproductive freedom. Finding resources for PCS was regarded as expensive and burdensome for Swedish healthcare system. To reach informed consent with expanded panels was also seen as a challenge. Furthermore, parents might perceive a pressure to undergo testing, if PCS was implemented. Finally, participants also expressed worries that PCS would increase medicalization and strive for control of pregnancy and parenthood. However, it was also mentioned that PCS might enhance reproductive autonomy and could reduce abortion incidence, since it allows parents to opt for reproductive decisions.

**Conclusion:** participants nurtured many ethical and non-ethical concerns regarding PCS that may affect the uptake and use. The results give insight of ethical concerns to consider, should PCS be implemented in Sweden.

#### EMP1.45

#### Discreditable inheritance: stigma and familial amyloid polyneuropathy ATTRV30M

**Á. Mendes<sup>1,2</sup>, L. Sousa<sup>3,4</sup>, J. Sequeiros<sup>1,5</sup>, A. Clarke<sup>6</sup>;**

<sup>1</sup>*i3S – Instituto de Investigação e Inovação em Saúde; UnIGENE and Centre for Predictive and Preventive Genetics (CGPP), IBMC – Institute for Molecular and Cell Biology, Univ. Porto, Porto, Portugal, <sup>2</sup>Department of Health Sciences, Univ. Aveiro, Aveiro, Portugal, <sup>3</sup>Department of Education and Psychology, University of Aveiro, Aveiro, Portugal, <sup>4</sup>CINTESIS, Center for Health Technology and Services Research, Univ. Aveiro, Aveiro, Portugal, <sup>5</sup>ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Univ. Porto, Porto, Portugal, <sup>6</sup>Institute of Medical Genetics, School of Medicine, Cardiff Univ., Cardiff, United Kingdom.*

Research on stigma related to genetic inherited disorders, although increasing, is still scarce. Here we report accounts of stigmatisation from patients affected by familial amyloid polyneuropathy (FAP) ATTRV30M living in Portugal's northwest coast, the largest cluster worldwide.

Semi-structured interviews were conducted with 11 patients, all mutation carriers at different stages after presymptomatic testing, recruited through the patients' association. Qualitative thematic analysis of the interviews was undertaken. Findings show the influence of a discrediting social context in the enactment of stigma: FAP was perceived as a source of devaluation and social distance, and permeated by beliefs of contagion in the community. The trans-generational nature of the illness was felt as a source of rejection for courtship and mating, and of devalued reproductive worth. Decisions to have children seemed to be a target of implicit negative judgment. Dealing with stigma included restraint in talking about FAP, especially outside the family, the active confrontation of others, and social withdrawal. Participants also refer a reduction in stigma over the past few decades, as medical information, potential treatments and clinical trials, are becoming more widespread. We discuss the interactional nature of stigma with individual biography, family dynamics, and societal changes.

These findings may be useful to set out the social consequences of stigma towards this group, and to understand how stigma is experienced in other genetic disorders for which presymptomatic testing is available. This is of importance to genetics and other healthcare professionals in assisting individuals to effectively manage these issues.

## KEYWORD INDEX

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